Introduction

Invasive plant species pose a serious threat to natural biodiversity in locales around the world (Callaway and Maron 2006, Mack et al 2000). While the biological and ecological processes of successful invasions are not fully understood, it is increasingly clear that these invasions can have significant impacts on ecosystems (Ehrenfeld 2003). Recently, much ecological research has focused on the biological invasions because they are affecting global change (Callaway and Maron 2006). By altering ecosystem functions and services, such as local and regional nutrient cycling and water filtration and cycling (Mack et al 2000), invasives, cost billions of dollars each year to the global economy (Pimentel et al 1999). Furthermore, the loss of biodiversity can cause ecosystem instability (Tilman et al 2006, Taylor et al 2006, Srivastava and Vellend 2005).

In order to fully understand the problems that invasive species cause, we must learn about the underlying biological and ecological processes that cause invasions. It is important, however, that we shape our understanding of invasive species within the context of specific invasions (Callaway and Maron 2006).

Community interactions are complex products of "huge numbers of interacting forces" (Clark and Gefland 2006). Indeed, the future of invasive species studies, Callaway and Maron (2006) suggest, is one that will have enhanced appreciation of multiple factors interacting to influence plant populations, community and ecosystem composition, and the role of invasive plants in those communities.

The complex relations and interactions between an ecosystem's parts create a delicate balance that can, however, be easily upset. Ecosystem degradation can be

provoked by the actions of humans, through means of agriculture, over-use and misuse of an ecosystem and other manners (Tilman 1999).

Streams, rivers and other flowing bodies of water are often particularly susceptible to anthropogenic degradation (Bernhardt et al 2005). The United States Environmental Protection Agency reports that nearly forty percent of surveyed rivers are polluted, and eight percent more are threatened by pollution (United States Environmental Protection Agency 2000). Rivers and streams play an important role in ecosystem processes. The riparian zone, the interface between dry land and flowing water, serves the role of water purifier and nutrient cycler (Kauffman et al 1997). Naturally occurring flora in the riparian zone prevent severe bank erosion and affect soil characteristics and water flow in the system (Kauffman et al 1997). Healthy riparian zones are important not only for the immediate areas, but also for ecological communities using the water downstream.

Areas of severe degradation are oftentimes subject to curative processes called ecosystem restorations (Srivastava and Vellend 2005). The primary goal of these restorations is to return a habitat to a more desirable state that includes a specific species and community composition to serve specific ecosystem functions (D'Antonio and Meyerson 2002). More specifically to streams and rivers, restorations attempt to return the water basins and riparian zones to a more natural, healthy state (Bernhardt et al 2005). The benefits of a successful restoration can be the reintroduction of nutrient cycling, water cycling and filtering, and improvement of water quality. Ecologically, riverine and riparian communities, both within, and downstream of, the restoration area, are revitalized and become more effective (Palmer et al 2005).

Although the standards of ecological success of these restorations are currently under debate (Palmer et al 2005), many of these projects have seen long-lasting positive ecological results. One means by which they may achieve less ecological success, however, is by facilitating the invasion of non-native plant species. Not only do invasives reduce the stability of the newly restored ecosystem, but they also affect the ecosystem function of the riparian zone (D'Antonio and Meyerson 2002).

Biological invasions are often problems in restoration sites. Invasive species tend to invade areas that have been recently disturbed (Callaway and Maron 2006, D'Antonio and Meyerson 2002), and stream restorations create massive disturbances. Invasives often form dense monoculture patches, dominating the invaded area. Many invasive plants are highly plastic, and effective at responding rapidly to small changes in the microclimate (Shea and Chesson 2002, Claridge and Franklin 2002). They are able to use this to their advantage by dominating and area before native species can respond to the changes. Hence, large-scale disturbances such as ecosystem restorations promote favorable conditions for invasion.

Stream restoration projects in North Carolina are many in number, and lofty in aspirations. The North Carolina Stream Restoration Institute is a large driving force for these efforts, and this has resulted in North Carolina having a relatively high proportion of its rivers restored (Bernhardt et al 2005). Many of the restoration sites, however, have been invaded by several exotic plants (personal observation, personal communication with Drs. Justin Wright, Emily Bernhardt, Chantal Reid, personal communication with North Carolina EEP office via Steven Roberts). We worry that these invasions may be affecting the ecosystem functions of restored streams.

One invasive plant of concern in these sites is *Microstegium vimineum*, commonly referred to as Japanese stiltgrass. *M. vimineum* is a C_4 grass that was introduced to the United States near Knoxville, Tennessee circa 1919 (Gibson et al 2002). At present, *M. vimineum* is found all over the Eastern United States, in the dense monoculture mats that are its common growth form (Gibson et al 2002). *M. vimineum* is a C_4 grass, yet it paradoxically is a shade-tolerant species (Cheplick 2006, Claridge and Franklin 2002). It is usually found in areas of recent disturbance, and often in mesic areas near water sources such as rivers, streams, lakes and temporally flooded lands (Horton and Neufeld 1998). Water–borne seed dispersal has been suggested (Gibson et al 2002), however this view is somewhat contentious (personal communication with Dr. Chantal Reid). *M. vimineum* is regarded as a high threat to natural species biodiversity (Judge et al 2005, Gibson et al 2002, Claridge and Franklin 2002, Horton and Neufeld 1998). The problem of invasion by *M. vimineum* is one of importance, especially in the setting of stream restorations.

My study of *M. vimineum* invasions at several stream restoration sites in central North Carolina focused on collecting and analyzing data in an attempt to correlate specific geographic, soil and environmental microclimate characteristics with invasions of the sites. In order to achieve these ends, field studies and laboratory techniques were used in combination. I hoped to show that geographic, environmental microclimate and soil characteristics would determine the extent of invasion and cover by *M. vimineum* at several stream restoration sites in central North Carolina.

The geographic characteristics that were tested include distance downstream from the head of the restoration project and lateral distance from stream bank. The seed

dispersal method of *M. vimineum* has been suggested to be by water-borne transport (Gibson et al 2002). Presumably, when a stream restoration takes place, the soil goes through enough removing and mixing that any prior existing *M. vimineum* seed bank in the soil is nearly, or completely, lost. Then, water borne seeds could only enter the system from upstream (no *M. vimineum* is intentionally planted in the restored sites), and they would land most frequently in the upstream part of the project. I hypothesized, therefore, to observe a negative relationship between distance downstream and *M. vimineum* cover. And, I expected to see a negative relationship between lateral distance away from stream bank and *M. vimineum* cover, as high water levels are less likely to reach the points further from the stream to deposit seeds.

The environmental microclimate was assessed by investigating canopy cover at the restoration sites. Canopy cover acts as a proxy measure for shade: higher values of canopy cover indicate more shady areas. *M. vimineum* has been shown previously to succeed in shaded areas (Claridge and Franklin 2002). It follows, then, that it may be more capable than other species in growing in shaded areas and can therefore out-compete them there. So, I hypothesized a positive correlation between canopy cover and *M. vimineum* cover.

Finally, the soil characteristics explored were soil moisture, soil organic content, and extractable soil nitrate. *M. vimineum* prefers moist growing areas, so I hypothesized a positive relationship between soil moisture and *M. vimineum* cover. More productive soils tend to have higher levels of organic matter, so they can support more growth. Therefore, I expected that soil organic matter levels would correlate positively with *M.*

vimineum cover. I expected that, as inorganic soil nitrogen is often a limiting nutrient for plants, *M. vimineum* would have a positive relationship with extractable nitrate.

Through the investigation of these varied characteristics, I hoped to gain a better understanding of what conditions affect *M. vimineum* invasion.

Methods

There were two important parts of the data collection of this experiment. First, geographical and environmental data was collected from the field, and soil samples were taken. Second, using lab procedures, the collected soil was analyzed for different soil characteristics.

The field data collection was done as follows. First, I selected four stream restoration sites in central North Carolina. Sites were selected to be of similar age and post-restoration planting treatment. At each suitable site, I planned for 25 plots, determined by a "stratified random" method. To do this, I measured 100 meters down from the head of the stream, placing a marker at each 20-meter interval. In each section I placed a transect perpendicularly across the stream, from one edge of the project to the other, where the placement of each transect was given by a random number generator. The distance of the transect was recorded from one end to the other. From this distance, I subtracted out the width of the stream, from bank to bank, then, I divided this distance into five equal length sections. Within each section along each transect I used a random number generator to determine where each plot would be placed. To see a visual representation of this method, please see Figure 1.

Once the plot placements were determined, each plot was visited and data recorded. At each plot site, a .5 x 1 m PVC rectangle was placed on the ground, centered about the determined point, with the long edge of the rectangle parallel to the stream. The percent cover of *M. vimineum* was recorded using visual estimation. To ensure fair and accurate visual estimation, estimates were double-checked by others until I felt comfortable that my estimates were accurate and consistent. Finally, using a spherical densiometer, I recorded canopy cover. I preferred to do multiple restoration sites on the same day to ensure unbiased data recording, however this was not always possible, but precautions were taken to minimize observational error. After recording data at each plot, I left a flag to mark the plot for returning for soil collection.

I did soil collections at all sites and plots on one day according to the following procedure. First, soil was collected on a warm, sunny day, with the last rain coming at least 3 days prior to collection. In order to take soil samples, first it was necessary to randomly select a sub-sample of plots at each site, due to time and resource constraints. Five plots at each of the four sites were randomly chosen for soil collection. This random selection led to choosing only three plots with any *M. vimineum*, and seventeen plots without it. Using a soil corer, the top 15 cm of soil at each chosen plot was taken and placed into a labeled, gallon-size Ziploc bag. Immediately, I closed each bag and placed it in a cooler full of ice in order to stop any microbial activity from occurring after the sample was taken.

In the lab, I determined soil moisture on a 10 ± 1 g sub-sample using a gravimetric technique. I then determined percent organic matter of the sub-sample using a loss on ignition (LOI) method (Robertson et al 1999).

To determine extractable nitrate, three different $10 \pm .5$ g sub-samples of each soil sample were placed into a 150 mL Erlenmeyer flask. Then, 100 mL of 1M KCl solution was added to each flask and each flask was shaken vigorously for five minutes by hand. Once all 60 sub-samples had been mixed thoroughly with KCl, all flasks were set on a shaker table for twenty-four hours to facilitate the removal of extractable nitrate from the soils. After this twenty-four hour period, the solution from each flask was collected. To do this, I shook each flask vigorously for one minute before pouring approximately 20 mL of the solution through a clean funnel with a clean filter paper into a clean, sealable glass vial. All solutions were treated in this manner, sealed in their vials, and placed in the freezer. Then, with the help of the Emily Bernhardt lab, each vial was analyzed for nitrate on a Lachat QuikChem® System.

I analyzed the collected data using several approaches. First, I analyzed the geographic and microclimate data by testing it with a linear regression against *M. vimineum* cover. I did this for each site individually, and all the sites combined. I also analyzed these data versus a presence/absence score of *M. vimineum*, using a logistic curve fit. With all soil data, I made linear regressions, using the average soil characteristics of a site and the sites' average *M. vimineum* covers to describe the sites. Because only three of twenty randomly selected plots had *M. vimineum* presence, I could only look at soil data as site average data. Presence/absence analysis were intended to give an indication of extent of invasion, while actual percent cover comparisons give an

indication of thoroughness of invasion. All analyses were performed in Microsoft Excel and JMP 6.0.

Results

Distance downstream from the head of the project, for all plots from all sites, showed no significant relationship with *M. vimineum* cover (Table 1). No singular sites, either, showed a significant relationship between distance downstream and *M. vimineum* cover (Figure 2). Using a nominal logistic fit for *M. vimineum* presence/absence with distance downstream, no significant relationship was established (Figure 3).

Lateral distance to stream bank, similarly, showed no significance when comparing this distance to *M. vimineum* cover for all plots from all sites (Table 1). Likewise, no individual sites showed significant correlations between distance to stream bank and *M. vimineum* cover (Figure 4). The nominal logistic fit for *M. vimineum* presence/absence with distance to stream bank does not show a significant relationship (Figure 5).

Canopy cover shows no significant relationship with *M. vimineum* cover for all plots across all sites (Table 1). Amongst individual sites, there were also no significant relationships between canopy cover and *M. vimineum* cover (Figure 6). In order to try to describe each site, wholly, I compared the average canopy cover for all plots of a site and the average *M. vimineum* cover in those plots. The linear regression that relates these values gives a positive relationship (Figure 7). The nominal logistic fit for canopy cover for all plots and *M. vimineum* presence/absence is a significant positive fit, but describes very little of the variation (Figure 8).

Soil data were analyzed as a linear regression of the average soil characteristic for each site versus average *M. vimineum* cover at that site. The relationship between soil moisture and *M. vimineum* cover was observed to be a negative, not significant relationship (Figure 9). Soil organic matter and *M. vimineum* cover showed a significant, positive correlation (Figure 10). Extractable nitrate and *M. vimineum* cover also showed a significant, positive correlation (Figure 11).

Discussion

Geographic data (distance downstream and distance to stream) were not significant predictors, suggesting that perhaps water-borne seed dispersal is not largely important in *M. vimineum*, or that prior seed banks affect invasion rates more than presumed. Microhabitat shade levels were not significant either, and perhaps indicate that other factors are more important. Soil data were the only data that had any significance, implying that soil data, especially soil organic matter and extractable nitrate at the site-wide level, can be predictors of *M. vimineum* invasion.

Contrary to what I hypothesized, geographic characteristics of plot sites did not have an impact on predicting or determining *M. vimineum* cover. While I assumed that water transported seeds would more likely germinate closer to the water source, this was not shown through my work. Neither distance to stream bank nor distance from head of project showed significant correlation with *M. vimineum* cover. They did not, either, suggest that such patterns may exist.

This may be explained by a number of theories. The first, that during the restoration process, the seed bank was not removed from the soil fully or effectively.

Any seeds that may have existed prior to the restorative process remained there afterwards, and readily germinated when the restoration process was completed. Because the removal of the seed bank of *M. vimineum* is not an explicit procedure, it is likely that this is, at least in part, contributing to the presence of *M. vimineum* at these stream restoration sites. In a long-term study, taking soil samples prior to stream restoration and examining them for *M. vimineum* seeds would give aid in determining whether seed bank prior to restoration was an important in predicting future invasion. Also, a comparison of pre- and post-restoration soil seed banks would give a better idea of how effective removal of *M. vimineum* seeds is during the restoration process.

Secondly, there could have been one or several floods between the time of the completion of the restoration and the time of data collection. One large flood could spread water-transported seeds throughout the riparian zone, leaving a broad area with a substantial *M. vimineum* seed bank. This would negate any relationship between distance to stream and *M. vimineum* cover that existed before a large flooding event. I could not find a reliable weather data history source to neither confirm nor deny this theory. This is a data that could be easily recorded in future, long-term studies.

Thirdly, *M. vimineum* may not transport seeds only through the means of water. Gibson et al (2002) suggest that this is the likely seed dispersal method. However, it was suggested to me by Dr. Chantal Reid (personal comment, based on her preliminary research) that *M. vimineum* does not rely solely on water-transportation methods for seed dispersal. Wind dispersal may be important, as a means of invasion from outside of restoration projects. The positive relationship between *M. vimineum* cover and distance to stream suggest that invasion from the outside in is a distinct possibility. Another study

might perform an exclosure experiment, comparing one open part of a restoration site to another closed one. This would help determine if outside seed inputs are important in determining *M. vimineum* invasion.

Given the highly plastic nature and large seed output of *M. vimineum* (Gibson et al 2002), it is likely that a combination of these three effects is responsible for the results found in my study. With further time and resources, I would suggest conducting a more thorough investigation of seed dispersal of *M. vimineum*. Seed bank, weather history and controlled exclosure experimentation would give a greater understanding of its invasion and ecological consequences.

The microhabitat characteristic of canopy cover proved to be, similar to the geographic characteristics, of little or no use in predicting or describing *M. vimineum* cover. This may be related to the competitive strategies of *M. vimineum*. Horton and Neufeld (1998) suggest that *M. vimineum* grows in low-light conditions and reacts quickly to absorb the high quantities of solar energy that come in short bursts of sunlight, called sunflecks. In another study, Barden (1996) showed that as light exposure increased on *M. vimineum*, it grew larger and heavier. So, while *M. vimineum* may be the most adept at growing in shade environments, it still remains a competitive factor in high-light microenvironments. So, factors other than canopy cover, the proxy for shade, likely have a more direct determination on the success of invasion by *M. vimineum* in a microhabitat.

One soil characteristic, soil moisture, did not show any meaningful relationship to *M. vimineum* cover, and did not prove to be a good predictor of it. This may be because, like light levels, *M. vimineum* prefers one type of growing environment, but is able to

compete in others as well. Like other invasives, *M. vimineum* is a highly adaptable plant that would likely be able to respond quickly to its environment and compete effectively. This would no doubt include soil moisture levels. Also, there are relatively small differences between average soil moistures of the soil collected. The physiological response to these small differences, does not vary as they would between a wetland and dry forest. In any riparian zone, soil moisture levels will be presumably similar, and comparing soil moisture between them is not very indicative. Another study may want to compare invasion in a wetland or riparian zone to a dry forest, where soil moisture differences will be more pronounced.

The remaining soil analyses indicate that soil characteristics may be a more important determinant of *M. vimineum* cover and success than any of the geographical or microclimatic traits. As average soil organic matter at a site increased, so did average *M. vimineum* cover. More productive soils, generally, have higher levels of organic matter. Coming back to the concept of plasticity, it is likely that *M. vimineum* is able to utilize this characteristic to out-compete native plants in the richest soils.

There is, though, another possible explanation. It is a possibility that *M. vimineum* contributes to high organic matter accumulation in these soils. It may lose leaves more frequently than other plants and this leaf litter could contribute to the increase in soil organic matter. This could be tested using leaf litter traps underneath *M. vimineum* plants and other species to determine which have the highest amount of leaf (or other organic matter) released. If *M. vimineum* did increase soil organic matter more than other plants, this too would explain the direct positive relationship between *M. vimineum* cover and soil organic matter. Kourtev et al (1999) raise questions about the quality of

organic matter in soils under invasives, so it may come from a different source (*M. vimineum*) than organic matter in other areas. An investigation of the litter from *M. vimineum* and other plants would provide data that could be compared with the data from Kourtev et al (1999) to determine possible sources of the organic matter.

Nonetheless, as my soil collection included very few plots with *M. vimineum*, it was a description of the site itself, not the plots with *M. vimineum*. So, it may be pre-existing high levels of soil organic matter that attracts *M. vimineum*. Further time and resources would be necessary to investigate the suggested experiments, which might resolve which alternative is more likely.

Kourtev et al (1999) report similar levels of extractable nitrate underneath *M. vimineum* as under native species in the same area. My data suggests a different trend; higher levels of *M. vimineum* occur in areas of higher nitrate. While my data set is, no doubt, limited, it still shows a very strong relationship between *M. vimineum* cover and soil nitrate. Further investigation would be necessary to determine whether my results are misleading because of their inadequate sample size, or whether they are different from what Kourtev et al (1999) found. I will continue here using the results from my study.

As with the soil organic matter, there is a question of causation: it is unclear whether *M. vimineum* causes high levels of nitrate in the soil underneath itself, or whether *M. vimineum* grows exceptionally well in areas of high soil nitrate.

Kourtev et al (1999) speculate that *M. vimineum* has "strikingly different chemical properties and biological activity" than native species, and would be the cause of the increased nitrate in the soil underneath it. Plant life histories are different and unique,

and this strategy may indeed be utilized by *M. vimineum*. To investigate this concern and determine causation, a study could look at the root exudates and leaf litter of *M. vimineum* and compare it to other plant species to see if the exudates and litter differ greatly in biochemical composition from other, indigenous plants. If they are similar, it is likely that the sites' soil characteristics determine the invasion, however, if they are greatly different, *M. vimineum* may cause the increase seen in extractable soil nitrate.

Because very few plots actually contained *M. vimineum* in my analysis, it suggests that the sites with higher nitrate levels in the soil are more attractive to *M. vimineum*. The sites that had more nitrate, as the correlation shows, also had more *M. vimineum*. Still, more investigation is needed to resolve this issue completely.

When looking at the soil data (moisture, organic matter and nitrate), it seems that Site 3 had dramatically higher *M. vimineum* cover than the other three sites. While it does not seem to pull the linear regressions enough to be an outlier, it is notable that Site 3 is so different. Site 3, I was informed while researching suitable sites, a restoration site with a long history of *M. vimineum* invasion. This site is also the most urban site, located in a park in the middle of Raleigh, North Carolina. Finally, the banks of the stream at this site were noticeably steeper than any other stream. Any or all of these factors may be important in the high levels of *M. vimineum* seen at this site. Investigating bank incline and aspect in relation to *M. vimineum* invasion could provide important predictive information for future stream restoration procedures. In sites with a history of invasion, it is extremely important that characteristics known to be implicit with *M. vimineum* are minimized.

While the results from this study may not give many significant predictors of what may lead to invasion by *M. vimineum*, it is useful to see where future studies should look when trying to understand invasions. Each characteristic tested here that does not have a significant relationship with *M. vimineum* cover does give further suggestions as to where new studies can and should look. These suggestions have been presented throughout this discussion, and can serve as a guide for future investigations. Any or all of them may be important to finding predictors of *M. vimineum* invasions, and furthering our understanding of the biological and ecological processes of said invasions.

Geographic characteristics seem not to play an important role in determining *M. vimineum* cover. They do suggest, however, that reassessing the notion that this invasive uses a water-borne seed dispersal method is desirable. This could lead to new beliefs about this plant species, and how invasions can be predicted or prevented. Canopy cover and soil moisture data, while not useful as predictors themselves, do reinforce the idea that *M. vimineum* is an adaptive and highly plastic plant species. Both soil organic content and soil nitrate level investigations in this study may help clarify the problem of causation surrounding invasives and their soil characteristics. So, while the conclusions may not be the intended or desired ones, they are important to our knowledge of *M. vimineum* as a plant and an invader.

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Tables and Figures

Table 1. This table shows the slope and R-squared values of the linear regression lines set to fit the "category" with *M. vimineum* cover for all plots from all sites. No relationships are found to be significant.

Category	Slope	R-squared
Distance to Stream	-0.996	0.028
Distance Downstream	-0.05	0.001
Canopy Cover	0.416	0.143
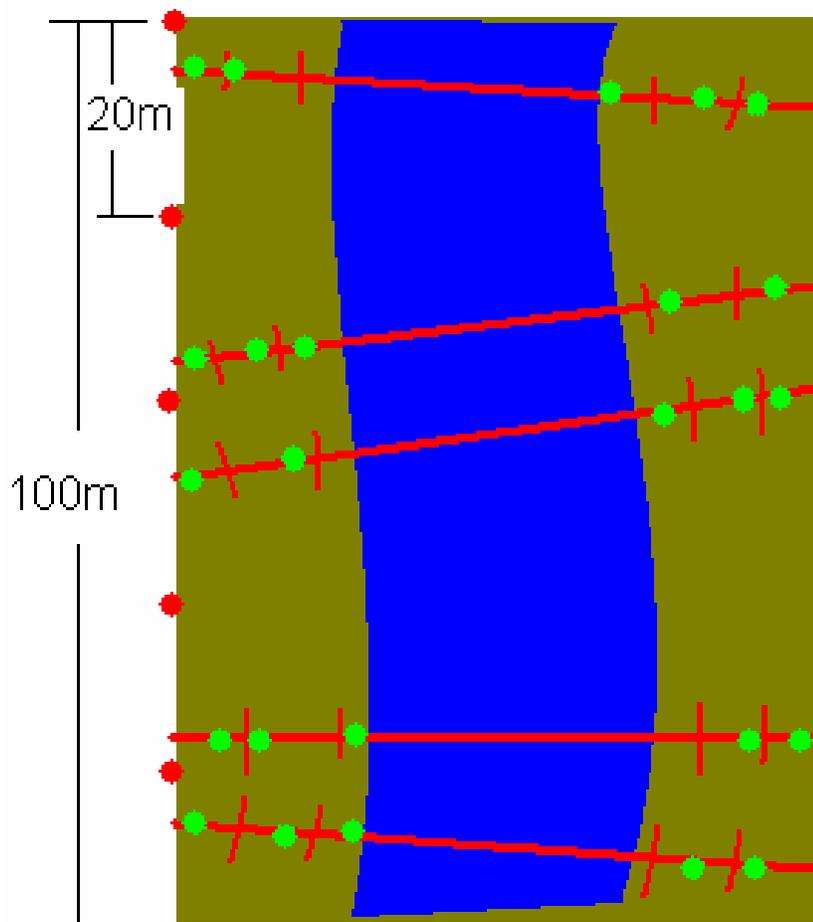
Figure 1. A visual representation of the "stratified random" method of plot selection to be used in the further experimental data collection. Each green dot indicates the presence of a plot. Notice that, generally, each area of the restoration project is represented, however there is no strict grid system which determines plot placement.
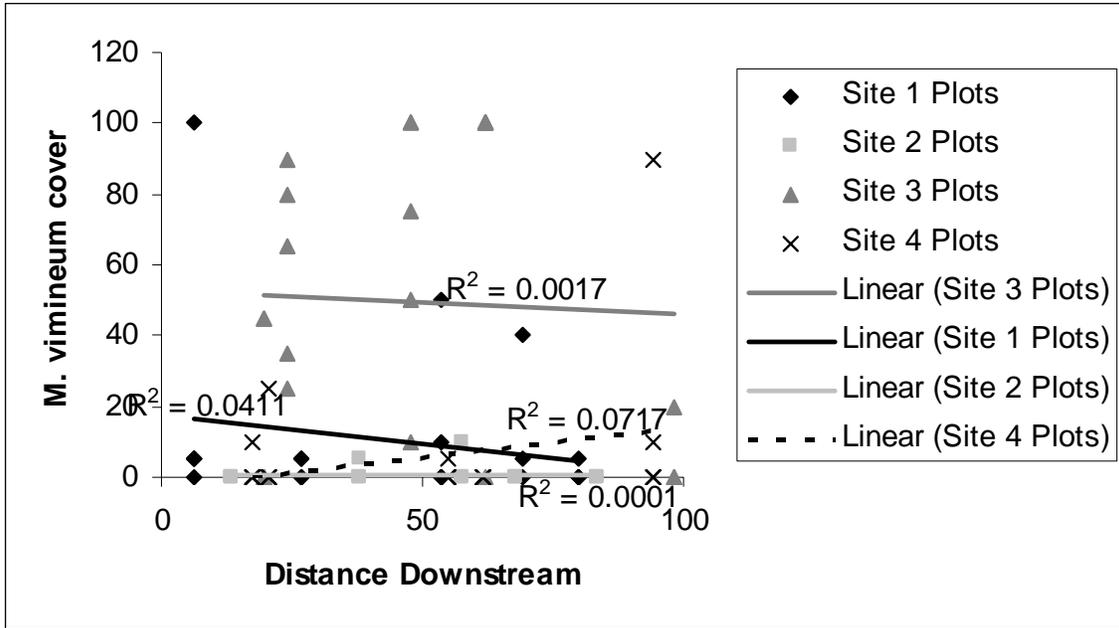
Figure 2. Linear regressions of *M. vimineum* cover versus distance downstream from head of restoration project for each site investigated. R-square values are shown for each regression. Site 1 and Site 3 show negative correlations, while Site 2 and Site 4 show positive correlations. All correlations are not significant.
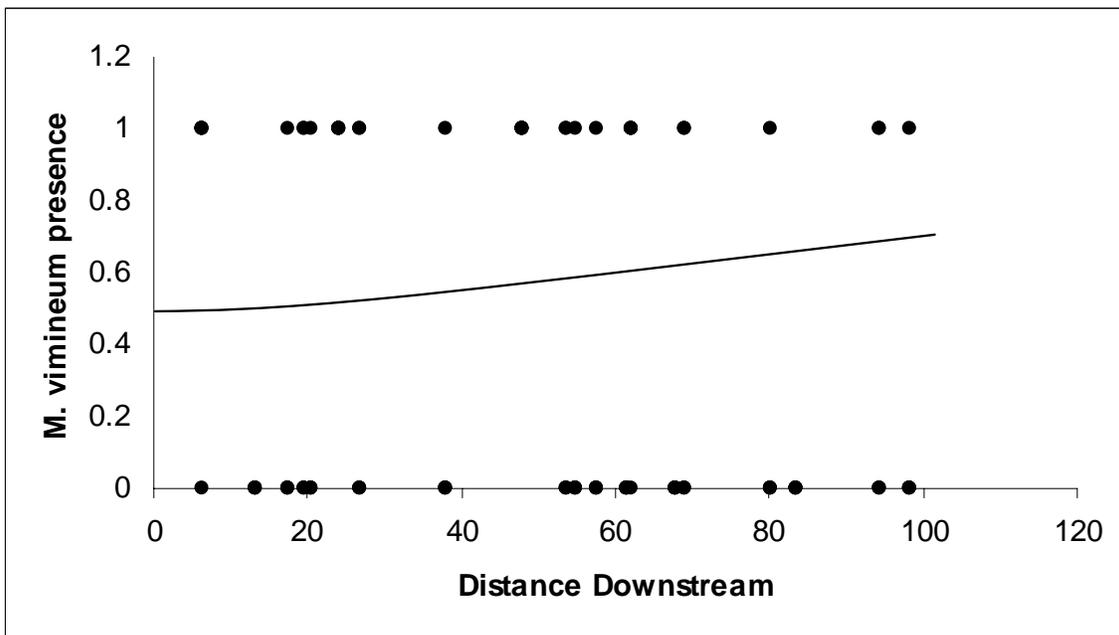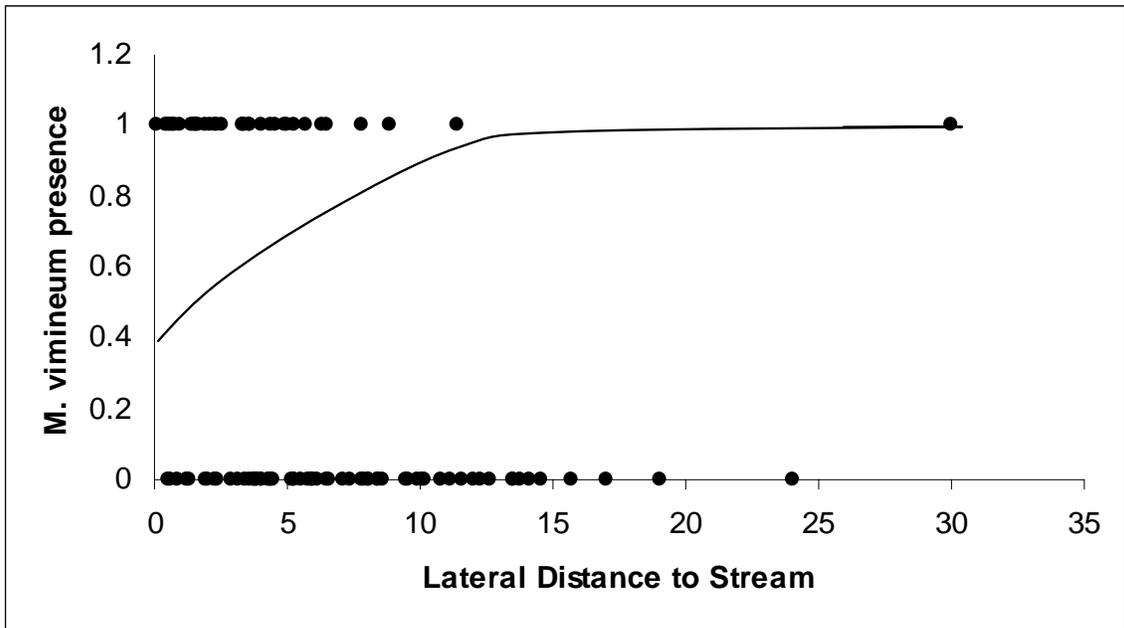

Figure 3. Nominal Logistic Fit for Presence/Absence of *M. vimineum* and distance downstream from the head of project. The relationship does not significantly describe any relationship (R-square = 0.0218).
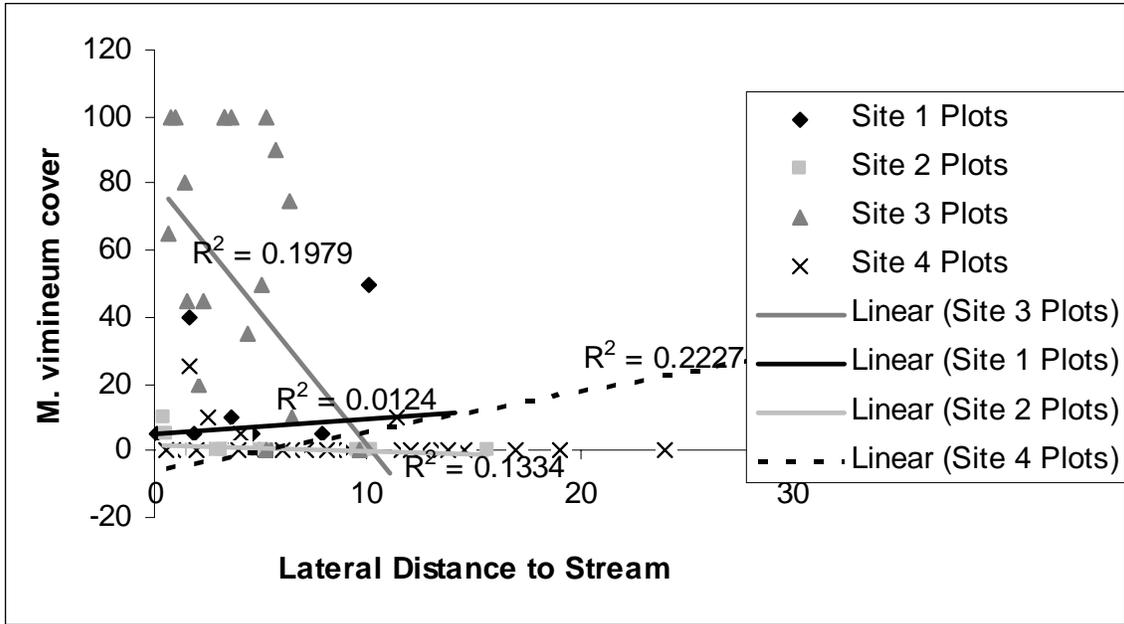
Figure 4. Linear regressions of *M. vimineum* cover versus lateral distance to stream for each site. R-square values are included for each regression. Site 2 and Site 3 each have negative correlations, while Site 1 and Site 4 each have positive correlations. All correlations are not significant.

Figure 5. Nominal Logistic Fit for Presence/Absence of *M. vimineum* and distance to stream bank. The relationship does not significantly describe any relationship (R-square = 0.0892).
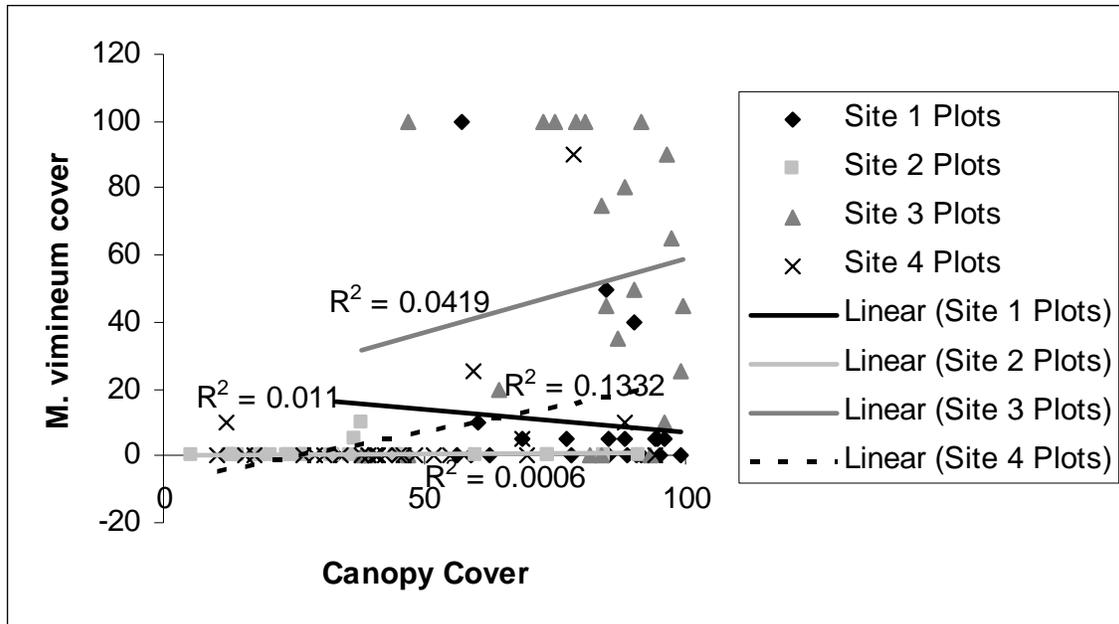
Figure 6. Linear regression of *M. vimineum* cover versus canopy cover for each site. R-square values are shown for each regression. Site 1 has a negative correlation, while Site 2, Site 3 and Site 4 all have positive correlations. None of the correlations are significant.
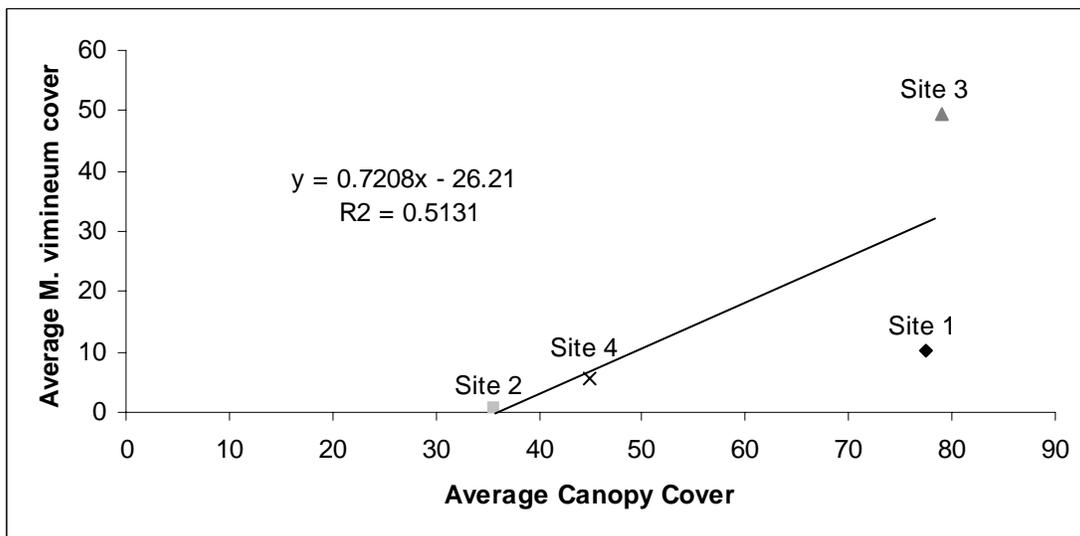
Figure 7. A linear regression giving the relationship between a sites' average canopy covers and average *M. vimineum* covers. R-square and linear regression formulae are shown on the plot.
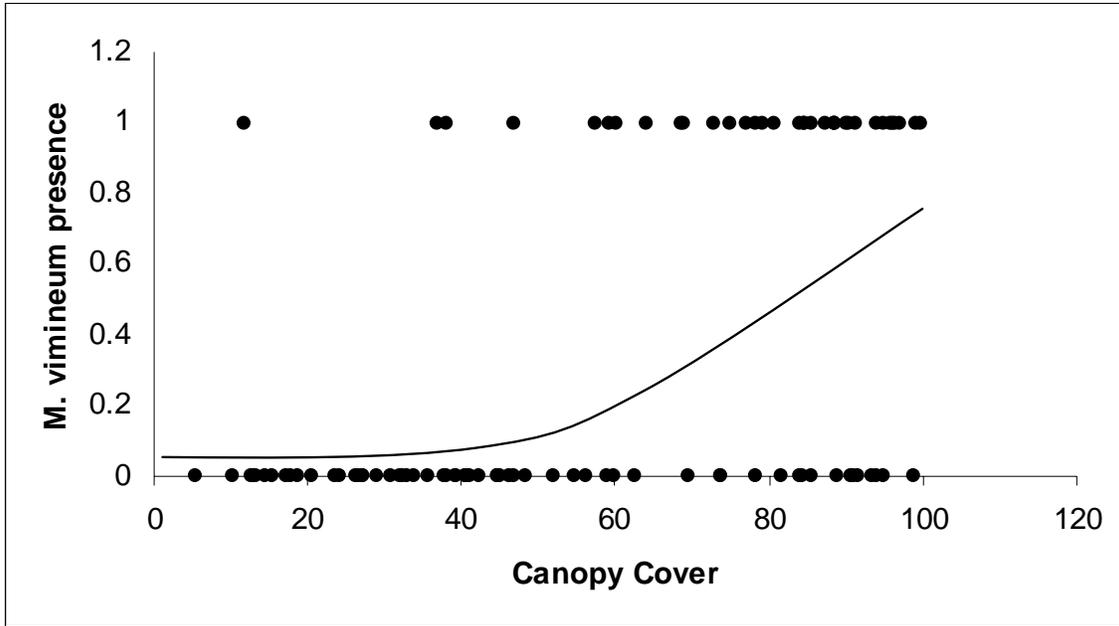
Figure 8. Nominal Logistic Fit for Presence/Absence of *M. vimineum* and canopy cover. The negative relationship is significant (p-value <.0001), however describes little of the variation (R-square = 0.222).
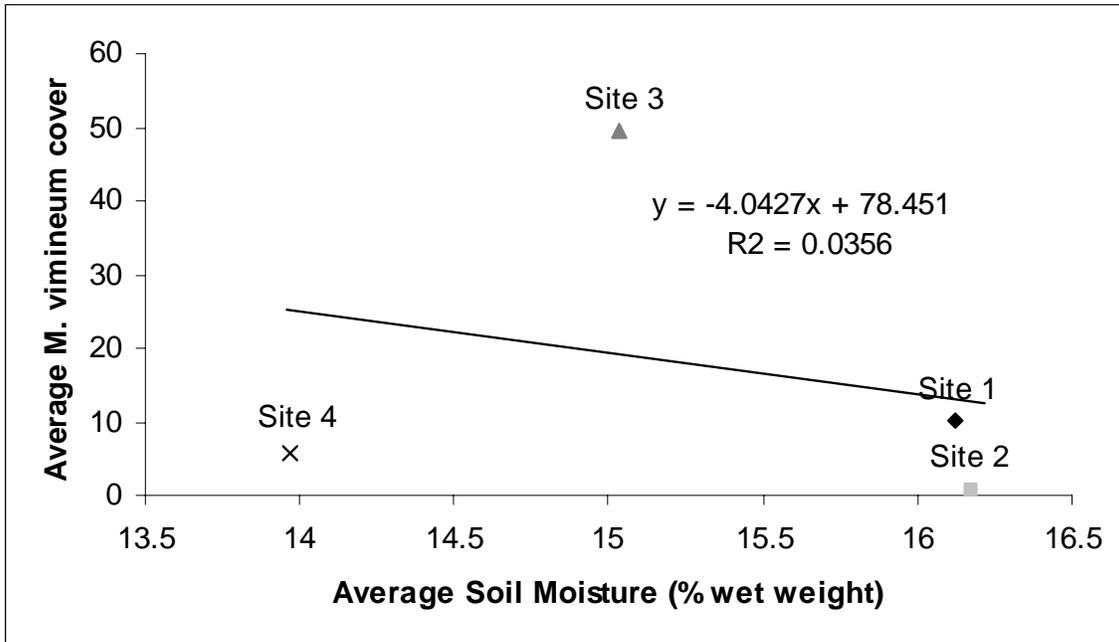

Figure 9. Linear regression for the average site-based soil moisture and *M. vimineum* cover. This negative relationship was not significant. R-square and regression line formula can be found on the plot.
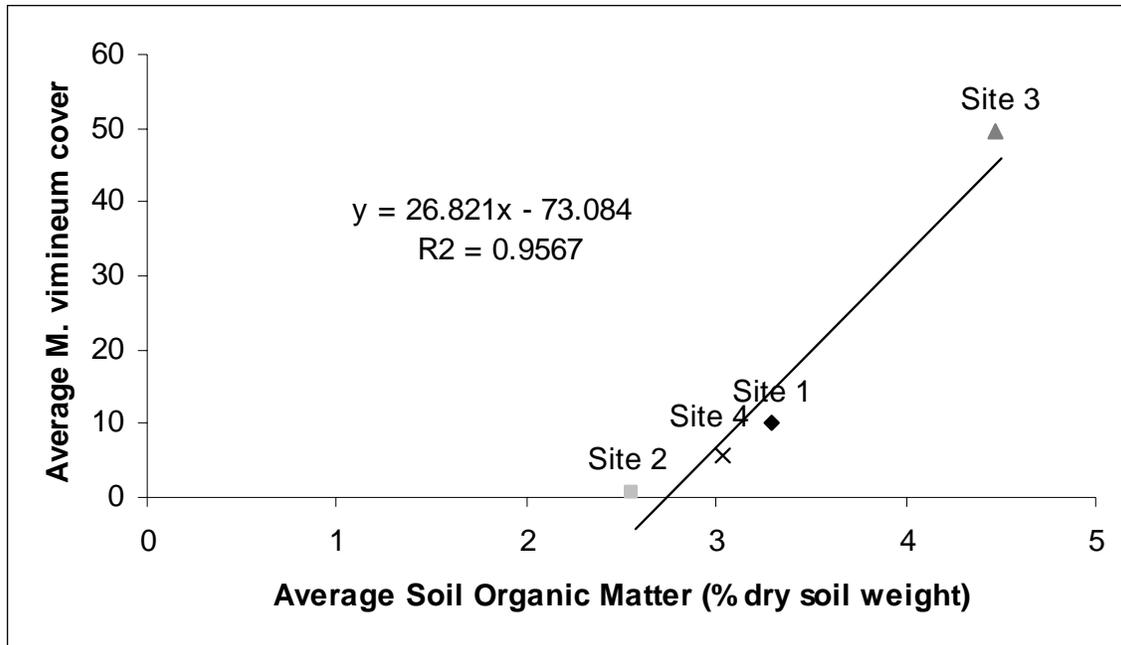
Figure 10. Linear regression for the average site-based soil organic matter and *M. vimineum* cover. This positive relationship was highly correlated for the four sites. R-square and regression line formula can be found on the plot.
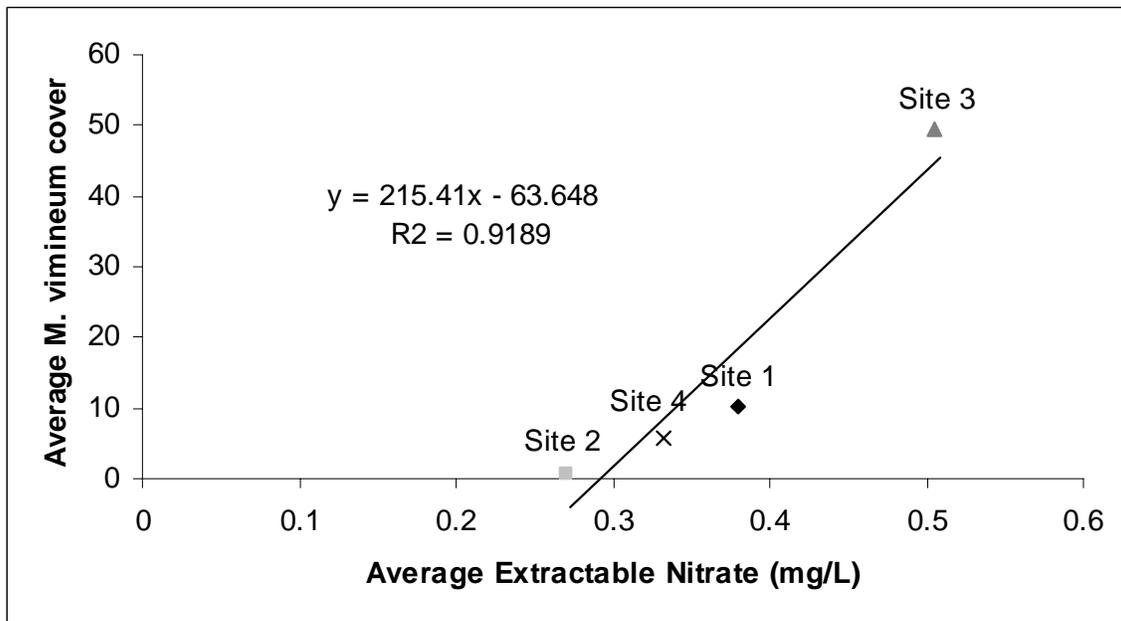

Figure 11. Linear regression for the average site-based soil extractable nitrate and *M. vimineum* cover. This positive relationship was highly correlated for the four sites. R-square and regression line formula can be found on the plot.