THE EFFECTS OF MICROSTEGIUM VIMINEUM ON THE SOIL MICROBIAL COMMUNITY

By

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ABSTRACT

Invasive species can spread into native ecosystems and become dominant species. The spread of invasive species is one of the largest threats to biodiversity. *Microstegium vimineum* is an annual C4 grass that is native to various parts of Asia. *M. vimineum* was introduced to the United States in the early twentieth century and has rapidly spread due to its high seed production. This study examined the effects of *M. vimineum* invasion on the composition of the soil bacterial community. Soil samples were collected from two woodland sites in northern Mississippi in November 2014 and 2015. The presence of *M. vimineum* was noted during 2015 sampling. DNA was extracted from each sample and next generation Illumina sequencing was used to sequence part of the 16S rRNA gene for the bacterial community. The soil bacterial community differed between 2014 and 2015 and between the two sites. A significant difference in the bacterial community was found between plots with and without *M. vimineum* at one site, but it was not seen at the other. While the results are suggestive of a difference in the soil bacterial community between plots with and without *M. vimineum*, more studies need to be performed to more fully determine its effect.
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INTRODUCTION

An invasive species is one that reproduces and rapidly spreads to nonnative ecosystems where it becomes a dominant species (Valéry et al. 2008). There are an estimated 50,000 invasive species in the United States with the number constantly increasing (Pimentel 2004). While some nonnative species, such as corn and wheat, are agriculturally important and have greatly contributed to the economy, other nonnative species have done more harm than good (Pejchar and Mooney 2009). At a global scale, the spread and impact of invasive species on an ecosystem is considered one of the greatest threats to biodiversity and ecosystem function (van der Putten et al. 2007).

A common invasive plant of the eastern United States is Microstegium vimineum (Japanese stilt grass), an annual C4 grass that is native to various parts of Asia. It was first discovered in the United States in 1919 in Tennessee (Gibson 2002). The plant spread rapidly and by 1960 covered many eastern coastal states along with Ohio and Pennsylvania (EPPO 2016). Today, M. vimineum has been recorded in 28 states and could be present in more. The rapid spread of M. vimineum is typically attributed to its high seed production. The US Forest Service has classified M. vimineum as a Category 1 invasive plant for the Eastern Region of the US, meaning that it is a highly invasive plant that is a threat to the native vegetation of an area (EPPO 2016).

An area of more recent interest in terms of invasive plant species is how they may influence the belowground or soil microbial community. Changes in plant species
composition can cause changes in the quality and quantity of root exudates to the soil (Ehrenfeld 2003, van der Putten et al. 2007), so it is likely that invasive plant species would alter soil properties. Invasive species can change the amount of organic matter in the soil compared to native vegetation (Ehrenfeld 2003), and invasive plants have also been shown to alter the rates at which nutrients cycle through the soil (Mack and D’Antonio 1998, Mack et al 2002, Faterrigo et al. 2010). While we are beginning to understand that invasive plant species can affect soil properties, little is known about the influence of invasive species on soil microbial community structure and function (van der Putten et al. 2007). Changes in the chemical and physical properties of the soil are likely to cause changes in the soil microbial community, which, in turn could change the functioning of the soil ecosystem.

The aboveground plant community influences the soil microbial community in a number of ways. Root inputs such as rhizosphere exudates along with the quantity and chemical quality of above ground litter, can lead to differences in community composition (Kourtev el al. 2003). Soil microbial communities also show seasonal variability that may be related to plant growing seasons, so that plant specific differences in soil community structure may occur (Smalla et al. 2001, Kourtev et al. 2002, Kulmatiski et al. 2008). However studies of the soil bacterial community around invasive plants tend to be descriptive, or are simple comparisons of the bacterial community of the invasive plant to the community around native vegetation. Native plant species are commonly displaced by invasive species yet the effect of this displacement on the soil microbial community is unknown (Kourtev et al. 2003).
In this study, I examined how the soil bacterial community changed following invasion by *M. viminalis*. By comparing the bacterial composition of soil plots over two years, I was able to examine how invasion by *M. viminalis* over this period specifically impacts the soil bacterial community.
METHODS

Study Sites and Sample Collection

Soil was collected from two sites at the Strawberry Plains Audubon Center in Holly Springs, Mississippi. The two sites were chosen in 2014 based on the presence of Microstegium vimineum at these sites. The trees at these sites included southern red oak (Quercus alba), hickory (Carya tomentosa), and post oak (Quercus stellate) (Brewer 2011). 25 samples were taken at each site, with specific sample locations chosen as being in the proximity of M. vimineum but not colonized by it in 2014 (i.e. sample location were selected based on a likelihood of colonization by M. vimineum over the next year). For each sample, 1.0-1.5 g of soil was collected from approximately 1 cm below the surface and placed in a 1.5 mL microtube using a spatula. The spatula was cleaned between each sample using an alcohol wipe. Latex gloves were worn while all soil samples were collected. After the collection, the soil was returned to the lab and stored at -20 °C until further processing. Soil was collected from each sample location on November 17, 2014 and again on November 10, 2015 for a total of 100 samples (2 sites x 25 samples x 2 years).

DNA Extraction and Sequencing

DNA was extracted from frozen soil samples using a PowerSoil DNA Isolation kit (Mo Bio Laboratories, Inc.) following the protocol provided. Approximately 0.25g of
soil was used for each extraction. Gel electrophoresis in 1.5% agarose gels was used to verify the presence of extracted DNA. The V4 region of bacterial 16S rRNA gene was amplified from the extracted DNA using a primer set optimized for Illumina MiSeq sequencing. The V4 region contains around 250 base pairs (Kozich et al. 2013). SequalPrep Plates (Life Technologies, Grand Island, NY) were used to normalize the concentration of amplicons, and all amplicons were combined into a single library. The library was sent to the Molecular and Genomics Core Facility of the University of Mississippi Medical Center for sequencing using an Illumina MiSeq system.

Data Analysis

Mothur, a bioinformatics software package, was used to analyze sequence data in the form of FASTQ files. The procedures recommended by Schloss et al (2011) and Kozich et al. (2013) were followed. After forming contigs and removing ambiguous bases, sequences were aligned to the SILVA rRNA database (Pruesse et al. 2007). The sequences were screened for chimeras using the uchime command and any chimeras were then removed (Edgar et al. 2011). All the remaining sequences were grouped into operational taxonomic units or OTUs using a 97% similarity between sequences. The sequences were then classified using the Greengenes database (DeSantis et al. 2006). All sequences that were classified as mitochondria, archaea, chloroplasts, or unclassified bacteria were removed.

To ensure that comparisons were not skewed by samples with low numbers of sequence reads, samples with less than 10,000 reads were removed. The remaining samples were normalized by subsampling to the read number of the sample with the
lowest read (10,323). Subsampling occurred over 1,000 iterations, and the mean scores and standard deviations were used to determine alpha-diversity (diversity of individual sample.) A rarefaction curve was generated to determine if the sequencing depth was sufficient. Beta-diversity (comparisons between samples) was assessed using the theta index, which checks the abundance of OTUs in each sample, and the Jaccard index, which compares samples based only on the presence or absence of OTUs. These two indices were used to generate non-metric multidimensional scaling (NMDS) ordinations to visualize the similarity of bacterial community structure between samples. An analysis of molecular variance (AMOVA) was performed to determine whether the difference between treatments was greater than the differences found within the treatments. The treatment used for analysis compared the presence of *M. vimineum* from 2014 to 2015. Finally, an analysis of similarity (ANOSIM) was performed which focuses on differences in community structure between treatments.
RESULTS

Presence of *M. vimineum*

During the November 2015 sampling, the proximity of *M. vimineum* to each sample point was noted. At Site 1, *M. vimineum* was within 30-61 cm in proximity at 7 out of 25 points. At Site 2, 13 out of 25 sample points were found to have *M. vimineum* 23-61 cm in proximity.

Bacterial Community Structure

A total of 3,910,447 sequences from 100 soil samples were obtained following Illumina 16S rRNA gene sequencing. The average sequence length was 253 base pairs. 45,782 sequences were identified as chimeras and were removed. The final valid bacterial dataset had 41,093 different OTUs when all samples were considered. In terms of phyla composition, the most dominant bacteria phyla were Proteobacteria (30.22% of the sequences), Planctomycetes (17.74%), and Acidobacteria (14.91%) (Figure 1).

The bacterial community showed significant differences in composition between 2014 and 2015. This significance was confirmed by AMOVA (p= 0.002, Fs= 1.669) and ANOSIM (p= 0.003, R= 0.062) for the Jaccard index. The theta index also showed significant differences when tested through AMOVA (p=0.0001, Fs= 3.905) and ANOSIM (p= 0.002, R= 0.065). NMDS plots showed these differences visually, whether ordinated based on Jaccard (Figure 2) or theta (Figure 3) dissimilarity indices. The mean
**Figure 1:** Dominant bacterial phyla found in soil communities at two woodland sites in northern Mississippi. The samples were taken at 50 different points in each of two years (2014, 2015) and characterized by sequencing of the 16S rRNA gene. Proportions are based on a total of 213,527 sequences.
(± standard deviation) number of OTUs found in each sample from 2014 was 2,052 ± 374, and the mean coverage (a reflection of the sampling depth) was 0.90. The mean (± standard deviation) invsimpson (measure of alpha diversity and indication of richness) in 2014 was 156 ± 49. The mean number of OTUs for the samples collected in 2015 was 2,011 ± 407 with a mean coverage of 0.90. The mean invsimpson for 2015 was 155 ± 56.

The soil bacterial community also proved to be significantly different between Site 1 and Site 2. This was confirmed by AMOVA (p < 0.001, Fs= 3.640) and ANOSIM (p < 0.001, R= 0.212) for the Jaccard index. The theta index showed similar results for AMOVA (p < 0.001, Fs= 8.776) and ANOSIM (p < 0.001, R= 0.174). NMDS plots further highlighted differences between Site 1 and 2. On the Jaccard NMDS plot, communities from Site 1 and Site 2 were clearly separated by the x-axis (Figure 2). The theta NMDS plot (Figure 3) had the largest grouping of samples from Site 1 in the upper right quadrant, and the largest grouping of samples from Site 2 in the lower left quadrant. The mean number of OTUs at Site 1 was 2,029 ± 481. The mean coverage at Site 1 was 0.90. The mean invsimpson was 164 ± 68 for Site 1. The mean number of OTUs at Site 2 was 2,033 ± 289, and the mean coverage was 0.90. The mean invsimpson at Site 2 was 148 ± 31.

When comparing the bacterial communities in sample points at Site 1 that had *M. vimineum* present in 2015 to those without, no significant difference was found whether tested through ANOSIM (p= 0.982, R= -0.187) or AMOVA (p= 0.749, Fs= 0.901) for the Jaccard index. Similarly, the theta index showed no significant difference in bacterial community structure at this site in relation to the presence of *M. vimineum* whether tested.
Figure 2: Jaccard NMDS plot shows difference in community composition of soil bacterial communities collected in 2014 (circle) and 2015 (triangle). Soil was collected at two woodland sites (Site 1 - red; Site 2 - blue).
Figure 3: theta NMDS plot shows difference in community composition of soil bacterial communities collected in 2014 (circle) and 2015 (triangle). Soil was collected at two woodland sites (Site 1- red; Site 2-blue).
by ANOSIM (p= 0.554, R= -0.038) or AMOVA (p= 0.591, Fs= 0.742).

Although not always statistically significant, the bacterial communities collected from sample points at Site 2 that had *M. vimineum* present were somewhat different from those without that plant. The ANOSIM (p = 0.187, R = 0.038) for the Jaccard index was the least statistically significant, but AMOVA results (p = 0.09, Fs = 1.156) suggested more differences between plots. When analyzed by the theta index, these differences were pronounced with ANOSIM being suggestive of differences between points with *M. vimineum* present and those without (p = 0.056, R = 0.067) and AMOVA indicating statistically significant differences (p = 0.016, Fs = 2.570).

Combining Sites 1 and 2, 20 out of 50 plots had *M. vimineum* growth in 2015. Two plots were removed due to low number of sequences, leaving 18 sample plots for analysis. For the Jaccard index, ANOSIM (p = 0.696, R = -0.028) and AMOVA (p=0.112, Fs= 1.143) did not show statistical significance between plots. Similar results were seen for the theta index for both ANOSIM (p= 0.419, R = -0.008) and AMOVA (p= 0.129, Fs= 1.559). The mean invsimpson for sample plots with *M. vimineum* present was 149 ± 47 and 159 ± 61 for plots without *M. vimineum* present.

The 16 most abundant OTUs, together, comprised 29% of the total number of reads (1,810,009 reads) with the most abundant OTU comprising 5.8% (105,495 reads) of the total. Four of the 16 most abundant OTUs (OTUs 1, 4, 13, and 15) were classified as Chthoniobacteraceae, a family of Verrucomicrobia. These four OTUs make up 179,527 or 9.9% of all valid reads. Another four of the 16 most abundant OTUs (OTUs 3, 8, 9, and 14) were unclassified Acidobacteria. Together these four OTUs comprise 111,439
reads or 6.2% of the total. OTUs 1, 2 and 3 were the three most abundant OTUs found at both Site 1 and Site 2. Similarly, OTUs 1, 2, and 3 were also the three most abundant OTUs in both 2014 and 2015. The same eight OTUs were found at sites with and without *M. vimineum*, however, the abundance of the OTUs varied between the sample plots (Figure 4).
Figure 4: Proportional abundance of operational taxonomic units (OTUs) in bacterial communities in soil collected from soil at 25 sites in each of the two north Mississippi woodland Sites. Comparisons are made between soil collected from the same sites in 2014 and 2015. (a), soil collected from the two sites (b), and soil collected from plots that had the invasive plant Microstegium vimineum present or absent (c).
DISCUSSION

In this study, I examined the structure of the soil bacterial community, and the effect of the invasive species *M. vimineum* (Japanese stilt grass) on the bacterial community. Overall, my results show a difference in the soil bacterial communities at Sites 1 and 2 and a difference between the bacterial community in 2014 and the bacterial community found in 2015. Importantly, at Site 2 there was a significant difference between plots with and without *M. vimineum*, however, this difference is not seen at the other site.

Proteobacteria, Planctomycetes, and Acidobacteria were the three dominant soil phyla among all the sample plots. Previous studies conducted using 16S rRNA gene sequencing have found that Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes, and Firmicutes are the nine phyla that make up approximately 92% of soil (Janssen 2006). Planctomycetes accounted for a larger percent of soil microbes than normally seen, but the percentages for Proteobacteria and Acidobacteria were found in common abundance.

The spread of invasive species is one of the greatest threats of biodiversity in an ecosystem (van der Putten et al. 2007), although the impacts of invasive plants on soil microbial communities has received only slight attention and have usually been addressed as comparison studies. Results of this study suggest that the presence of *M. vimineum* has an effect on the soil bacterial community. Bacterial species richness was
lower at sample plots with *M. vimineum* than at plots without *M. vimineum*. A decrease in the biodiversity of the soil could have repercussions on the native species. Changes in species richness that occur as a result of the addition of invasive species and the loss of native species alter the ecosystem processes (Ehrenfeld et al. 2001). If the bacterial species necessary for native plants to thrive are diminished or completely removed, native plants could die out allowing the invasive species to fully take over the area.

Reductions in soil biodiversity could also affect rates of carbon or nutrient cycling. *M. vimineum* prefers NH$_4^+$ and NO$_3^-$ over organic sources of nitrogen, has a higher C:N shoot ratio, and distributes a greater amount of N to above ground biomass than native species (Fraterrigo et al. 2011). *M. vimineum* invasions have been found to cause an increase in soil nitrogen cycling, soil pH, and reduce rates of litter decomposition (Ehrenfeld et al. 2001; Kourtev et al. 2002, 2003), all of which could be included by changes in bacterial community structure and diversity.

A significant difference in bacterial community structure between sample plots with and without the presence of *M. vimineum* was only found at Site 2, although the same patterns were suggested at Site 1. The significance at Site 2 and not Site 1 could be because of the greater presence of *M. vimineum* at Site 2 (13 out of 25 points) compared to Site 1 (just seven out of 25 points). This could have allowed the effects of *M. vimineum* to be more pervasive or just facilitated the greater statistical detection of differences. Regardless of the presence of the invasive plant, the bacterial communities at Sites 1 and 2 were significantly different from each other. It is possible that the bacterial community at Site 1 was not as susceptible to change from invasive colonization as that at Site 2. Determining if invasive plants have the same impact in different
locations, even within the same geographic area, has rarely been addressed, yet it is an important question from both an ecological and conservational viewpoint.

While overall differences were found in the community structure between sites, the same OTUs were dominant at both Sites 1 and 2. This suggests that the differences between the sites could be caused by differences in less common OTUs. When comparing OTU abundance between plots with and without the presence of *M. vimineum*, the same OTUs were dominant for both cases. The greatest difference between sample plots with and without *M. vimineum* was the percent abundance of OTU02. Its presence was greater at plots without the presence of *M. vimineum*. OTU02 was identified as a member of the Bradyrhizobiacea, a family of Alphaproteobacteria. The specific genus found was *Bradyrhizobium*, which is important in nitrogen fixation (Marcondes de Souza et al. 2014). This bacterium may not have been as abundant in plots containing *M. vimineum* as *M. vimineum* does not form symbiotic associations with nitrogen fixing bacteria, but as mentioned above, primarily uses NH$_4^+$ and NO$_3^-$ as its sources of nitrogen.

Further studies would help develop a deeper understanding of how *M. vimineum* truly affects the soil bacterial community. One limitation of my study was only having two years of data. This study could be further developed by gathering samples from the same plots over multiple consecutive years. This would allow more time for *M. vimineum* to potentially invade other plots, increasing the number of comparisons to be made, and further invade areas it was already present, potentially increasing any effect that it may have. Tracking the spread of invasive species over many years would be the ideal method of examining their effects, but this method involves a commitment of time.
and resources that may not be possible. Examining the invasion of *M. vimineum* in other ecosystems across the US could also allow for greater comparison of the effects.


