PLANT SPECIES COEXISTENCE: THE ROLES OF SOIL BIOTA AND INVASION HISTORY

by

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ABSTRACT

Plant soil feedbacks, specifically soil microbial communities, have been proposed to affect plant species’ relative abundances and stabilize coexistence within a community. Negative plant soil feedbacks, where plant species condition microbial communities detrimental to conspecifics, may promote coexistence and plant diversity within a community both by lowering overall fitness and creating negative frequency dependence, i.e. stabilizing niche differences. Positive plant soil feedbacks, where plant species condition communities beneficial to conspecifics, may promote the dominance of a species through increased overall fitness and reduced negative frequency dependence. Invasive species can disrupt coexistence and reduce resident species abundance and diversity in its invaded range, potentially due to a more neutral to positive interaction with the soil microbial community resulting from an escape from specialized pests and pathogens in its native range. However, a more antagonistic soil community could accumulate through time to reduce the dominance of the invader and promote coexistence between it and native plant species. We explored whether the propensity to coexist increased due to the presence of soil biota, as well as whether the likelihood of coexistence increased across invasion history for invasive Microstegium vimineum and native plants due to changes in the soil
community. In an observational study across *M. vimineum*’s invasive range in the eastern United States, we found a decline in *M. vimineum*’s survival at low frequency and changes in its soil/root fungal community across invasion history. In a 2-year field experiment negative frequency dependence increased for *M. vimineum* across invasion history in conspecific conditioned soil. Finally, in a greenhouse experiment we found that the soil community promoted coexistence of *M. vimineum* and native *Pilea pumila* through increased stabilizing niche differences. These results combined show that the soil community promotes coexistence through stabilizing interactions and that the likelihood of coexistence between invasive *M. vimineum* and native plants is potentially increasing through invasion time due to an accumulation of an antagonistic soil community.

**INDEX WORDS:** coexistence, stabilizing niche differences, average fitness differences, plant, soil, pathogen, microbe, invasion, per capita population growth rate, frequency dependence
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CHAPTER I: INTRODUCTION

Contemporary coexistence theory

Recent advances in coexistence theory have broken down coexistence into two components, stabilizing niche differences and average fitness differences (Chesson 2000). Stabilizing niche differences are differences between species that promote coexistence by limiting conspecifics more strongly than heterospecifics (Chesson 2000, Adler et al. 2007), while average fitness differences between species promote competitive exclusion (Chesson 2000). Stabilizing niche differences result in negative frequency dependence for a species, i.e. decreasing per capita population growth rate with increasing conspecific frequency (Chesson 2000). Negative frequency dependence promotes coexistence through a higher per capita population growth rate when a species is rare within a community, discouraging species extinction, and lower per capita population growth rate when a species is abundant relative to other species within a community, discouraging competitive exclusion. Average fitness differences are overall differences in demographic vital rates (e.g. survival, fecundity, and growth) or response to competition that are independent of species’ relative abundances within the community (Chesson 2000). The larger the average fitness differences are between species the larger the stabilizing niche differences have to be to stabilize coexistence. The limiting case of equivalent average fitness and no stabilizing niche differences produces Hubbell’s neutral model, in which coexistence and community assembly are random processes (Hubbell 2001, Adler et al. 2007). Multiple biotic
and abiotic factors affect average fitness differences and/or stabilizing niche differences (HilleRisLambers et al. 2012).

In classical plant ecology theory, coexistence and the relative abundance of plant species within communities have been explained by resource competition (Grime 1979, Tilman 1982). Species dominance or rarity is driven by their ability to acquire resources relative to other species and coexistence is stabilized when species differ in their resource requirements or the space and timing of their resource acquisition (Grime 1979, Tilman 1982). More recently, plant-soil microbe interactions have been proposed as a mechanism for coexistence, due to their ability to drive negative frequency dependence within plant species (Bell et al. 2006, Bever et al. 2010, Bagchi et al. 2014) and their correlation to plant species’ relative abundances (Bever et al. 1997, Klironomos 2002, Kulmatiski et al. 2008, Mangan et al. 2010, MacDougall et al. 2011).

Soil microbial communities may affect the two components of coexistence in several ways. For instance, specialized pathogens can exert negative frequency dependence when disease transmission correlates with host density, leading to a higher probability of epidemics in dense populations (Burdon and Chilvers 1982). Alternatively, access to soil mutualists may increase the average fitness of one plant species over another, reducing the likelihood of coexistence. Plant species can alter the assembly of soil microbial communities in their root zone, and these legacies provide another source of stabilizing or destabilizing effects. Negative plant soil feedbacks, where plant species condition microbial communities detrimental to conspecifics, may promote coexistence and plant diversity within a community both by lowering overall fitness and by creating negative frequency dependence, as germinants are more likely to encounter detrimental soils when their conspecifics are at high frequency (Kulmatiski et al. 2008). Positive plant soil feedbacks, where plant species condition communities beneficial to
conspecifics, may promote the dominance of a species, or even destabilizing priority effects, by the creation of positive frequency dependence (Bever et al. 2010, van der Putten et al. 2013).

**Plant invasions and the disruption of coexistence**

When invasive species enter communities they often disrupt species coexistence and are associated with declines in resident species diversity and abundance (Vila et al. 2011). Invaders’ superior competitive ability (Vila and Weiner 2004) and plant community dominance could be due to their lack of coevolutionary history in the invaded range (Hallett 2006). The advantage, through evolutionary novelty, could come in the form of novel weapons (Hierro and Callaway 2003, Callaway and Ridenour 2004, Zheng et al. 2015), unique niche requirements (Lloret et al. 2005, Godoy et al. 2009, Brym et al. 2011), or enemy release (Mitchell and Power 2003, DeWalt et al. 2004, Liu and Stiling 2006). However, whether this advantage persists through invasion time is unclear; ecological or evolutionary forces could develop to reduce competitive exclusion and promote coexistence between native and invasive plants (Strayer et al. 2006, Lankau et al. 2009, Iacarella et al. 2015).

Since the soil microbe community may promote coexistence through negative plant-soil feedbacks, invader dominance may arise due to a tendency for invaders to have neutral or positive plant-soil feedbacks (Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004). A more positive plant-soil interaction in the invaded range could be due to an absence of specialized enemies or novel mutualistic interactions (Reinhart and Callaway 2006). However, plant-soil feedbacks for nonnative species have been shown to become more negative across time since establishment (Diez et al. 2010, Dostal et al. 2013).

A soil microbe community with a short invasion history may lack the specialized pests and pathogens present in the native range of the invader (Reinhart and Callaway 2006). A more
positive plant-microbe interaction could promote competitive exclusion by the invader through weak negative frequency dependence and high average fitness relative to native plants in the community. A soil microbe community with a long invasion history has potentially accumulated specialized pests and pathogens (Hawkes 2007, Mitchell et al. 2010, Schultheis et al. 2015) causing for a more negative plant-soil feedback (Diez et al. 2010, Dostal et al. 2013). A more negative plant-soil microbe interaction could promote coexistence between the invader and the native community through increased stabilizing forces, i.e. negative frequency dependence, and reduced average fitness.

**Dissertation research**

Through my dissertation I explored whether soil biota promoted coexistence between *Microstegium vimineum* and native plants and whether this changed across invasion history. In Chapter II, I used an observational field study to assess whether *M. vimineum*’s frequency dependence, fitness at low frequency, and soil fungal community were changing across invasion history. In Chapter III, I conducted a 2-year field experiment exploring changes in competitive interactions between *M. vimineum* and native *Pilea pumila* across invasion history and whether changes were due to conditioning of the soil by the invader. In Chapter IV, I performed a greenhouse experiment to examine whether the likelihood of coexistence between *M. vimineum* and *P. pumila* increased in the presence of invader conditioned soil biota and whether this increased across invasion history of the soil biota.

**Study system**

*Microstegium vimineum* is an Asian, C4 annual grass that has aggressively invaded forest understories throughout the eastern United States. It was first collected in its invasive range in 1919 near Knoxville, TN (Fairbrot.De and Gray 1972). It is capable of establishing in a variety
of habitats and has a broad environmental tolerance (Cole and Weltzin 2004), although it is commonly found in moist habitats such as floodplains, stream banks, and riparian areas (Barden 1987, Redman 1995). It is unusual for a C4 plant since it is shade tolerant and commonly found in understory environments (Winter et al. 1982, Horton and Neufeld 1998), although deep shade limits its establishment and reproduction (Cheplick 2005, Cole and Weltzin 2005, Schramm and Ehrenfeld 2010, Warren et al. 2011).

Typical of an aggressive invader, *M. vimineum* has been found to have a negative effect on native flora and fauna. *Microstegium vimineum* reduces native plant biomass and diversity (Oswalt et al. 2007, Adams and Engelhardt 2009, Flory and Clay 2010a) and suppresses tree seedling regeneration (Marshall et al. 2009, Flory and Clay 2010b). Higher trophic levels are also negatively affected by *M. vimineum* invasion with decreases in abundance and diversity of arthropods as well as shifts in trophic interactions of forest floor consumers (McGrath and Binkley 2009, Simao et al. 2010, DeVore and Maerz 2014). *Microstegium vimineum* has also been found to shift belowground nutrient cycling and microbial communities (Kourtev et al. 2003, DeMeester and Richter 2010, Fraterrigo et al. 2011, Strickland et al. 2011).

The reasons for *M. vimineum*’s invasion success are not clear, but multiple mechanisms have been proposed including evolution post invasion (Flory et al. 2011), alteration in nutrient cycling (Ehrenfeld et al. 2001, Lee et al. 2012), and disturbance (Glasgow and Matlack 2007, Eschtruth and Battles 2009, Kuebbing et al. 2013).

**References**


CHAPTER II:

DECLINING SURVIVAL ACROSS INVASION HISTORY FOR *MICROSTEGIUM VIMINEUM*

1 Cunard C and Lankau R. To be submitted to Biological Invasions.
Abstract

Many introduced species become invasive because they lack coevolutionary history with the native species in the invaded range; particularly they lack specialized enemies. Lack of enemies could enable the invader to establish and persist when rare within a new community, and lead to dominance by the invader through exponential population growth. However, could this advantage degrade through invasion time as specialized pests and pathogens accumulate? We investigated survival rates and individual biomass as proxies for per capita population growth rates for the invasive annual grass, *Microstegium vimineum*, across 12 sites that varied in estimated time since invasion. We also explored changes in the belowground fungal community associated with *M. vimineum* across this chronosequence. *Microstegium vimineum*’s frequency dependence changed from negative to neutral across the invasion time gradient and the shift was driven by a decline in survival at low frequency. Changes in the root and soil fungal community were associated with time since invasion and possibly pathogen driven. These results suggest that *M. vimineum* may be less prone to persist at older invaded sites, due to an accumulation of pathogens in the soil community, and thus may be more vulnerable to management intervention.
Introduction

Many introduced species gain an advantage over the native species due to a lack of coevolutionary history with the invaded community. This evolutionary advantage could come in the form of novel weapons like plant allelochemicals, escape from specialized pests and pathogens, or unique niche requirements (Hallett 2006). These advantages could allow invasive species to establish and then outcompete native species, and indeed invasive plant species are commonly observed to competitively exclude native plants from local communities (Vila et al. 2011), even if they do not cause range wide native extinctions (Sax et al. 2002).

However, it remains unclear whether the dominance by invasive species will be stable over time or shift to coexistence between the invader and native species. This could occur if the invasive species’ evolutionary advantage degrades through time as both the invasive and native species evolve post-introduction (Lankau et al. 2009, Dostál et al. 2013). Specifically, pathogen accumulation could reduce this advantage (Flory and Clay 2013) and larger pathogen loads have been found on plant species with older introduction dates (Hawkes 2007, Mitchell et al. 2010).

In order to establish in a new community, an introduced species must have a positive population growth rate when rare (i.e. at low conspecific frequency) (Chesson 2000, Adler et al. 2007). Furthermore, high per capita population growth rate when rare will act to buffer the invader’s population from local extinction, and promote spatial spread through the local area. On the other hand, if the invader’s per capita population growth rate is low (zero or negative), then the population will not be able to increase from its initial founding, and will be vulnerable to extinction when population density declines.

While per capita population growth rate at low frequency determines whether an introduced species can establish, its ultimate density depends on frequency dependence. Whether
an invasive plant has a stabilized or an exponentially growing population depends on how its per capita population growth rate changes with conspecific frequency. An invasive plant that has positive frequency dependence, increasing per capita population growth rate with increasing frequency of conspecifics, would have exponential population growth. On the other hand, an invader with negative frequency dependence would have a more stabilized population since per capita population growth rate decreases with increasing frequency of conspecifics.

Invasive species that dominate their introduced habitats do so through some combination of high per capita population growth rates at low frequency and weak negative (or even positive) frequency dependence. Escape from specialized enemies may allow invaders to have these traits since enemies can have overall negative effects on per capita population growth rates and frequency dependence. Higher specialized pathogen loads could increase negative frequency dependence, potentially leading to stable coexistence between the invader and native competitors. An accumulation of pathogens could also reduce population growth rates at low frequency, which would potentially make it more difficult for the invader to persist in the community. Furthermore, a decline in per capita population growth at low frequency could also make the population more vulnerable to management intervention.

The primary objective of this study was to investigate whether there was evidence for changes in invasive Microstegium vimineum’s frequency dependence and individual plant performance at low frequency (as a relative proxy for per capita population growth rate in this annual species) across invasion history. We predicted that M. vimineum’s frequency dependence and performance at low frequency would be negatively correlated with time since invasion. We chose to study M. vimineum because it has invaded a wide area in eastern N. America mainly
through natural dispersal (rather than intentional planting), and has a simple annual lifestyle that allows a more direct link between individual performance and population dynamics.

The enemy release hypothesis has not been tested on *M. vimineum*, however aboveground accumulation of fungal pathogens that decrease *M. vimineum*’s performance have recently been reported in the invasive range (Flory et al. 2011a). In this study, we were specifically interested in the accumulation of belowground pathogens. Plant-soil feedbacks can become more negative with increasing time since invasion (Diez et al. 2010, Dostál et al. 2013) and this could affect the invader’s per capita population growth rate and frequency dependence. Therefore, our secondary objective was to identify any changes in the belowground fungal community that were correlated with invasion history and potentially affecting individual performance and frequency dependence of *M. vimineum*. We predicted that there would be changes in the soil/root fungal community associated with invasion history that would be suggestive of pathogen accumulation.

**Methods**

**Study system**

*Microstegium vimineum* is a C4, Asian annual grass. It was first collected within its invasive range in the United States in Knoxville, TN in 1919 (Fairbrot.De and Gray 1972). It is a shade tolerant invader in eastern forests (Winter et al. 1982). *Microstegium vimineum* reduces the richness of native herbs (Flory and Clay 2010a), suppresses tree seedling regeneration (Flory and Clay 2010b), and is associated with changes in nutrient cycling (Frattirrego et al. 2011, Strickland et al. 2011). Multiple mechanisms have been proposed for *M. vimineum*’s invasion success, including evolution post invasion (Flory et al. 2011b), alteration in nutrient cycling (Ehrenfeld et al. 2001), and disturbance (Glasgow and Matlack 2007, Kuebbing et al. 2013).
Map of invasion history

We created a map of _M. vimineum_ invasion history across the eastern US using ArcMap in ArcGIS 10.1 (Fig. 2.1). We created a database of _M. vimineum_ collection dates and locations by contacting herbaria across the eastern US. We divided the eastern US into a 0.1 latitude by 0.1 longitude grid and assigned the oldest collection date to each cell, totaling 542 points. If a cell did not have an assigned date we left it blank. Using these data we created a spatial kriging layer of invasion history.

We used ordinary kriging to interpolate estimated invasion ages based on our herbarium records. We used ordinary kriging, which assumes there is a constant trend between distance and the relationship between points, because we had no scientific explanation for using a model in which the trend varies across the landscape (Environmental Systems Research Institute 2013). We used an exponential model to describe the spatial autocorrelation between points, which we determined as the model of best fit using the semivariogram (Environmental Systems Research Institute 2013). The kriging layer used the simplest model of invasion history, which excluded anisotropy (directional spatial autocorrelation), and had the appropriate lag size (43,500) and lag number (20), equaling about half of the largest distance between points (1,740,000 m) when multiplied together. The layer used the standard search neighborhood, with a maximum number of 5 points and a minimum number of 2 points within each of the 4 sectors that were at a 45° offset, to calculate predicted values from points. The average difference between the measured and predicted values was 0.0403. The standardized mean prediction error was 0.0019, which is appropriately close to 0 (Environmental Systems Research Institute 2013). The root mean squared standardized was 0.9586 which is close to 1, meaning the prediction standard errors are valid. The root mean squared prediction error was 15.8590 and indicates how close the model
predicts the actual point values. The average estimated prediction standard error was 16.6453. Since the root mean squared prediction error and the average estimated prediction standard error were similar and the root mean squared standardized was close to 1, we concluded that the kriging layer was appropriate (Environmental Systems Research Institute 2013).

Site and plot design

We then used the map to choose 12 sites that varied in *M. vimineum* time since invasion (Fig. 2.1). The sites ranged from an estimated invasion age of approximately 11 to 49 years. We sampled at each of these sites twice, once at the end of May (spring/s) and then at the end of September (fall/f) 2012. At each site we established 12 plots in an area invaded by *M. vimineum*, except for sites CNF and BEF where we only established 7 plots due to the small size of the invasion. We randomly designated 1m² plots along a transect of the invaded area. Eight invaded and 4 uninvaded plots were established at each site; at CNF and BEF there were 4 invaded and 3 uninvaded plots. We randomly selected the first 6 invaded plots and then, if necessary, intentionally chose the last 2 invaded plots to try to maintain a range of percent cover of *M. vimineum*. We did this at each site so we could explore frequency dependence within sites and how frequency dependence changed across sites. In the study, we had a total of 134 plots.

At all 12 plots at each site we measured variables that could potentially affect individual plant performance, frequency dependence, or be associated with time since invasion. At both sampling time points, within each plot we took percent cover estimates of 4 categories, grass/sedge, woody, herbaceous, and *M. vimineum*. We measured % canopy openness over each plot using hemispheric photography done with a digital camera (Canon EOS Rebel T3) with a fish eye lens (Opteka). The camera was held level 1m above the center of the plot. We analyzed the photos using Gap Light Analyzer software (Frazer et al. 1999).
We measured available nitrate (NO$_3^-$) and ammonium (NH$_4^+$) in each plot by placing 20 g wet mass of mixed bed ion exchange resin (Rexyn™ 300 (H-OH) Beads (Analytical Grade/Certified), Fisher Chemical) in the field for the duration of the study (~4 months). Bags for the resin were made of nylon stockings cut into 10 cm squares and zip tied closed. We charged the resin bags with 0.5 M HCl and rinsed them with DI water until the pH was neutral. We buried the resin bags at a depth of ~10 cm within each plot. We collected the bags during the fall sampling, rinsed them with DI water, and individually extracted them with 100 ml of 2M KCl. Extracts were analyzed for NO$_3^-$ and NH$_4^+$ with a continuous flow colorimetric assay at the Analytical Chemistry Laboratory at the University of Georgia. Our final sample size was 119 (78=invaded, 41=uninvaded) due to loss.

We collected soil from each plot and oven dried it. From each site we combined equal amounts of the 8 invaded plot samples (4 for BEF and CNF) and separately combined equal amounts of the 4 uninvaded plot samples (3 for BEF and CNF), resulting in 12 invaded and 12 uninvaded soil samples for processing. We had a routine nutrient test performed at the University of Georgia Soil, Plant, and Water Laboratory. The test provided measurements of soil pH and extractable phosphorus (P), potassium, calcium, magnesium, manganese, and zinc. We also analyzed total % nitrogen (N) and carbon (C) and calculated the C:N ratio of the oven-dried soil. For the C and N analysis, we ball-milled the soil samples to less than 250 um particle size and weighed 24-26 mg into 5 x 5 mm tin capsules. The capsules were analyzed with Micro-Dumas combustion at the Analytical Chemistry Laboratory at the University of Georgia.

Molecular analysis

We collected *M. vimineum* roots, during the spring sampling, and soil, from both sampling time points, from each plot to perform terminal restriction fragment length
polymorphism analysis (T-RFLP) of the general fungal and arbuscular mycorrhizal fungal (AMF) community. We explored the AMF community as well as the general fungal community so we could have the ability to rule out the AMF community as the particular functional group driving any patterns we observed in the general fungal community. Having the ability to rule out AMF as the drivers of specific patterns allowed us to more strongly infer that patterns were being driven by other fungal functional groups such as pathogens. Soil was collected from the top 10 cm within each plot and trowels were sterilized with 70% ethanol in between each plot. We collected fine roots from *M. vimineum* individuals in all invaded plots. We stored all of the samples for molecular analysis on ice until returning to the lab where we stored them in a -80°C freezer.

We extracted DNA from all materials using Omega Bio-tek (Atlanta, GA) extraction kits. For the general fungal community, the polymerase chain reaction (PCR) targeted the internal transcribed spacer (ITS) region of the ribosomal RNA gene segment using primer pair ITSf1 and ITS4r (Koide and Dickie 2002, Laurent et al. 2008). For the AMF PCR, the small subunit ribosomal RNA region was targeted using primer pair AML1 and AML2 (Lee et al. 2008). The PCR protocols followed those in St. Laurent et al. (2008) for general fungi. The PCR protocols for AMF were from Lee et al. (2008), except 0.5g of T4 gene 32 protein (Roche Diagnostics) was added to the AMF PCR and PCR cycles were increased to 35. The forward primers were labelled with 6-FAM fluorescence (Operon Biotechnologies, Inc., Huntsville, AL, USA) to allow us to detect fragments with capillary electrophoresis.

After PCR, the products were digested with a restriction enzyme; Hha for general fungi and Mbo for AMF (Promega). Capillary electrophoresis on an ABI Prism 3730xl DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) sized the fragments using a fluorescent lane standard,
ROX1000. We then used GENEMAPPER v 3.0 (Applied Biosystems, Carlsbad, CA, USA) for size-calling of the fluorescence peaks using two basepair allele bins. Peak areas, roughly equivalent to the relative abundance of the operational taxonomic unit (OTU) represented by that fragment, were used for all analyses.

**Estimating frequency dependence**

During the spring sampling, we randomly tagged 5 *M. vimineum* individuals in each invaded plot (n=88) by zip tying them to metal stakes. In the fall sampling, we collected the aboveground biomass of all individuals present, oven dried them at 60°C, and weighed their final biomass. Individuals that were gone from their marked spot were recorded as dead. We calculated the proportion of survivors and average living biomass (g) for 82 of the invaded plots, losing 6 plots that were not relocated. The frequency of *M. vimineum* within each plot was calculated by dividing *M. vimineum* % cover by the total % cover of all plants.

**Statistical Analyses**

To test whether *M. vimineum* frequency dependence changed with time since invasion, we regressed the proportion of survivors or the average living biomass of *M. vimineum* individuals within each plot against *M. vimineum* frequency (s/f), time since invasion, and their interaction. We ran a generalized linear model with a binomial distribution when proportion of survivors was the dependent variable and a multiple linear regression when it was average living biomass. If we found a significant interaction within a model, we also used the estimate of the time since invasion main effect to understand how survival/living biomass at low frequency was associated with time since invasion, since the estimate represents how time since invasion is associated with the variable at 0/low frequency.
We also tested whether frequency dependence was changing across other variables including latitude, total soil N, soil P, % canopy openness, available NO$_3^-$, available NH$_4^+$, and soil C:N. To explore significant interactions between frequency dependence and time since invasion or any other significant environmental variable we chose the highest and lowest frequency plots from each site. We used linear models to test whether *M. vimineum* survival/living biomass changed across time since invasion or other environmental variables in only high frequency or only low frequency plots. We ran models separately for each significant variable and also included each environmental variable and time since invasion in the same model. All models had *M. vimineum* frequency (s/f) as a covariate.

Using a permutational MANOVA through the adonis function in the vegan statistical package in R version 3.2.1, we tested whether changes in the general fungal/AMF communities in the soil and roots were associated with time since invasion. The fungal community OTU’s proportional abundances for each plot were averaged for each site to run these analyses separately for roots (spring) and invaded and uninvaded soil (spring and fall), with n=12 for each analysis. We performed these analyses for uninvaded soil and the AMF community in order to rule these groups out as potential drivers of changes in the fungal community. We also tested whether fungal community composition was associated with the other environmental variables that significantly affected frequency dependence.

**Results**

Frequency dependence changed with time since invasion when the proportion of survivors was used as the dependent variable. This was shown by a significant interaction between *M. vimineum* frequency (s/f) and time since invasion (s, f respectively: LRT=5.0864, 7.9760, P=0.0241, 0.0047, Table 2.1). Survival decreased with increasing frequency of *M.*
vimineum at younger invaded sites and was similar across all frequencies at older invaded sites (Fig. 2.2 & 2.3), due to a decline in survival at low frequency across invasion time. The estimate for the main effect of time since invasion was negative for both spring and fall (s, f respectively: -0.0562, -0.0854, Table 2.1) and survival was decreasing across time since invasion at low M. vimineum frequency in both spring and fall (s, f respectively: LRT=6.5453, 7.6847, P=0.0105, 0.0056, Table 2.2 & 2.3) and increasing at high frequency in fall only (LRT=5.5005, P=0.0190, Table 2.3). The negative relationship between survival and time since invasion at low M. vimineum frequency was maintained when we included other environmental variables that affected frequency dependence in the model, but this was not true for the positive relationship between survival and time since invasion at high frequency (Table 2.2 & 2.3). The relationship between frequency dependence and invasion time was not significant when we used average living biomass as the dependent variable.

Frequency dependence changed across latitude, total N, soil P, and available NO$_3^-$ when we used proportion of survivors as the dependent variable (Table 2.1, Figs 2.4-2.7). This was shown by a significant interaction between M. vimineum spring frequency and each variable (Table 2.1). The interaction with M. vimineum fall frequency was only significant for total N, soil P, and NO$_3^-$ (Table 2.1). Survival was declining across each of these variables at high M. vimineum frequency (s/f), but there was no trend at low M. vimineum frequency (s/f) (Table 2.2 & 2.3).

Changes in the spring general fungal community on M. vimineum roots were associated with time since invasion (P=0.0400, R$^2$=0.1481), but not with latitude, soil P, total N, or NO$_3^-$. Changes in the root AMF community were not associated with time since invasion (P=0.5045,
R²=0.0754). Also, changes in the general fungal community in spring invaded soil were not associated with time since invasion (P= 0.5734, R²= 0.08047).

Changes in the general fungal community in fall invaded soil were associated with time since invasion (P=0.0210, R²=0.1438), but not any other environmental variable (latitude, soil P, total N, or NO₃⁻). These changes associated with time since invasion were not found for fall uninvaded soil (P=0.5774, R²=0.0813). Changes in the AMF community in fall invaded soil was not associated with time since invasion (P=0.3576, R²=0.1003).

**Discussion**

Over time invasive species may lose the ecological advantages they gain due to their evolutionary novelty in a community (Lankau et al. 2009, Dostál et al. 2013). We proposed that this phenomenon would manifest in population dynamics of the invader: namely, that over time invasive populations would develop stronger regulation, via more negative frequency dependence, and/or reduced ability to persist when rare. Our results support the latter prediction, but not the former, and suggest that over time *M. vimineum* populations may become more prone to local extinction. The decrease in survival at low frequency could not be explained by co-varying environmental gradients, but patterns in fungal communities on *M. vimineum* roots are consistent with potential increases in pathogen loads over time.

Frequency dependence of survival shifted from strongly negative to neutral with increasing time since invasion across our 12 sites. Contrary to our hypotheses, these results provide no evidence for accumulation of population stabilizing forces. At young invaded sites survival was negatively correlated with frequency of *M. vimineum*, suggesting negative frequency dependence. At older invaded sites the relationship was neutral with similar survival across all frequencies, suggesting neutral frequency dependence. However, this neutral pattern
resulted from low survival across all frequencies. *Microstegium vimineum*'s seedling survival to reproduction is an important component of life-time fitness, affecting its recruitment ability and thus the per capita population growth rate (Warren et al. 2013). Survival to reproduction is especially important at low *M. vimineum* density due to the necessity of having individuals present to produce seed and maintain the population. *Microstegium vimineum*'s survival at low frequency was negatively correlated with time since invasion, as we hypothesized. This suggests that *M. vimineum*'s per capita population growth rate at low frequency is declining through invasion time, decreasing its ability to persist when rare within a community (Chesson 2000, Adler et al. 2007). This pattern was supported with both fall and spring estimates of *M. vimineum* frequency. We did not observe this significant pattern when we used average living biomass as the fitness measure. This suggests that whatever is driving the change in frequency dependence is taking place at earlier stages of development, rather than during biomass accumulation.

Although the negative frequency dependence of *M. vimineum* survival at more recently invaded sites suggests population stabilization, this does not rule out the possibility of it being a dominant invader. It is still quite possible for its population to reach high density and frequency before it becomes stabilized. If the per capita population growth rate does not become negative until higher frequency than *M. vimineum* could be a dominant member of the community. Due to the scope of our study we cannot determine this, but this study does suggest that *M. vimineum* has more potential to be a dominant community member at young invaded sites, while at older invaded sites this is less likely due to constant lower survival rates.

The strength and direction of frequency dependence was also associated with latitude, total N, soil P, and available NO$_3^-$.

For the three soil nutrient variables, frequency dependence was neutral at low nutrients and then negative at high nutrients, driven mainly by a decline in
survival rates at high frequencies in higher nutrient sites. This may have resulted from greater self-thinning at more productive sites. However, these relationships were sensitive to one or two highly productive sites (Figs 2.5-2.7). A decline in survival at high frequency may not harm *M. vimineum*’s population since conspecifics could compensate for the loss in seed production. Frequency dependence was neutral at low latitudes and negative at higher latitudes. This pattern was driven by survival at high frequency decreasing at higher latitudes. A shift in the relative importance of intraspecific resource competition (leading to self-thinning at high frequency) relative to natural enemies (leading to low survival at all frequencies) from high to low latitudes could explain this pattern (Schemske et al. 2009), although we found no evidence for changes in the soil fungal community related to latitude. Importantly, the pattern of decreasing survival at low frequencies with increasing time since invasion was not confounded by these environmental gradients, which predominately affected survival at high frequency.

One possible mechanism for the decline in *M. vimineum*’s survival with invasion time is an accumulation of soil pathogens. We observed a change in the general fungal community on *M. vimineum* roots across invasion time but not specifically in the AMF community. Arbuscular mycorrhizal fungi are usually considered to have a mutualistic relationship with plants, although they are capable of being parasitic (Johnson et al. 1997). Other fungal guilds in the root fungal community include pathogens, saprotrophs, and endophytes, and it is unclear which of these groups were driving fungal community changes across time. We did not find similar changes in the bulk soil community during the spring sampling, suggesting that the changes were caused by fungi more specific to *M. vimineum*, whether mutualistic or pathogenic. However, we did find that the fall bulk soil general fungal community composition correlated with invasion history. Changes in the fall fungal community were exclusively found in invaded soil, which suggests
that the changes are \textit{M. vimineum} specific. This suggests the differences in the root fungal community were prevalent earlier during the growing season, but these specific fungal species’ populations did not spread into the surrounding soil community until later in the season.

Our results suggest management of \textit{M. vimineum} will be more successful at older invaded sites. Multiple management options are used against \textit{M. vimineum} including herbicides, fire, and physical removal (Ward and Mervosh 2012), all with the goal of eradicating or reducing invasion. All options would be more effective at older invaded sites due to the decline in \textit{M. vimineum}’s survival (and likely per capita population growth rate) at low frequency. At younger invaded sites, interventions that reduce \textit{M. vimineum}’s population to low frequency within the community may be counteracted by \textit{M. vimineum}’s high per capita population growth rate when rare. On the other hand, a reduction in \textit{M. vimineum}’s population may lead to local extinction due to low survival at low frequency at older invaded sites. Future studies that examine \textit{M. vimineum} control across time since invasion could benefit management efforts.

Our results are consistent with other studies that suggest the invasive advantage, gained from lacking coevolutionary history with the invaded community, can degrade through invasion time (Lankau et al. 2009, Dostál et al. 2013). Our data demonstrate changes in the relationship between survivorship and frequency for \textit{M. vimineum} across invasion time that would benefit native plant species in the community. Although our study does not define a clear mechanism for the decline in survivorship of \textit{M. vimineum} across time, our data suggests that belowground pathogen accumulation could be a driver, which is consistent with studies on other species (Hawkes 2007, Diez et al. 2010, Mitchell et al. 2010, Dostál et al. 2013). Our study adds to the current literature by focusing on the invader’s key vital rates across invasion time, which will ultimately determine population dynamics and persistence. Understanding how an invader’s
population dynamics change through invasion time is vital to predict its long-term impact and design the most effective management strategies.

References


Frazer, G. W., C. D. Canham, and K. P. Lertzman. 1999. Gap light analyzer (GLA), version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation Simon Fraser University, Burnaby, British Columbia and the Institute of Ecosystem Studies, Millbrook, NY


Table 2.1- Statistics for general linear models with binomial distribution and proportion of *Microstegium vimineum* survivors as the dependent variable (n=82, except for models that include nitrate (NO$_3^-$) where n=77). Testing for the significance of the interaction between *M. vimineum* spring (s) and fall (f) frequency and multiple explanatory variables. ** = significant at the p=0.05 level, *** = significant at the p=0.001 level. Total N = total % soil nitrogen, soil P = extractable soil phosphorous (ppm), available NO$_3^-$ = soil available nitrate over the growing season (µg/gram of resin bead)

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Table 2.2- Statistics for general linear models with binomial distribution and proportion of *Microstegium vimineum* survivors as the dependent variable (n=12). Using only the highest or lowest *M. vimineum* frequency (freq) plot from spring for each site. * = significant at the p=0.1 level, ** = significant at the p=0.05 level, *** = significant at the p=0.001 level. Total N= total % soil nitrogen, soil P= extractable soil phosphorous (ppm), nitrate= soil available nitrate over the growing season (µg/gram of resin bead).

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Table 2.3 - Statistics for general linear models with binomial distribution and proportion of *Microstegium vimineum* survivors as the dependent variable (n=12). Using only the highest or lowest *M. vimineum* frequency (freq) plot from fall for each site. * = significant at the p=0.1 level, ** = significant at the p=0.05 level, *** = significant at the p=0.001 level. Total N= total % soil nitrogen, soil P= extractable soil phosphorus (ppm), nitrate= soil available nitrate over the growing season (µg/gram of resin bead).

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Figure 2.1 - Map of *Microstegium vimineum* invasion history, created using the kriging function in ArcMap from ArcGIS 10.1. Points on map are the 12 sites where the study was performed and are also listed in the table with their abbreviated identification, state of location, and estimated time since invasion.
Figure 2.2- Contour plot of the proportion of Microstegium vimineum survivors across *M. vimineum* spring frequency and time since *M. vimineum* invasion of each of the 12 sites.
Figure 2.3- Contour plot of the proportion of *Microstegium vimineum* survivors across *M. vimineum* fall frequency and time since *M. vimineum* invasion of each of the 12 sites.
Figure 2.4- Contour plot of the proportion of *Microstegium vimineum* survivors across *M. vimineum* spring frequency and latitude of each of the 12 sites.
Figure 2.5- Contour plot of the proportion of *Microstegium vimineum* survivors across *M. vimineum* spring frequency and extractable phosphorous (P) of *M. vimineum* invaded soil at each of the 12 sites.
Figure 2.6- Contour plot of the proportion of *Microstegium vimineum* survivors across *M. vimineum* spring frequency and % total nitrogen of *M. vimineum* invaded soil at each of the 12 sites.
Figure 2.7- Contour plot of the proportion of *Microstegium vimineum* survivors across *M. vimineum* spring frequency and average available nitrate of *M. vimineum* invaded soil at each of the 12 sites.
CHAPTER III:

STABILIZING FORCES, DRIVEN BY CONSPECIFIC CONDITIONED SOIL,
ACCUMULATE ACROSS INVASION HISTORY FOR MICROSTEGIUM VIMINEUM

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Abstract

While many plant communities are species rich, invasive species can drive reductions in local species diversity and abundance. Invasive species may be superior competitors in their invaded range due to their lack of coevolutionary history with the native community, resulting in less negative plant-soil interactions due to a release from specialized pests and pathogens. A less negative plant-soil interaction could promote the invader’s dominance through reduced stabilizing forces, i.e. reduced negative frequency dependence, and/or increased fitness relative to native plants. However, it is unclear whether the invasive advantage persists through invasion time. Through a two year field experiment we explored whether estimates of the two coexistence components, average fitness differences and stabilizing niche differences, changed for invasive Microstegium vimineum and native Pilea pumila across time since M. vimineum invasion and whether this depended on conditioning of the soil by the invader. We found that stabilizing niche differences, i.e. negative frequency dependence, increased for M. vimineum across time since invasion and this pattern was more evident in soil conditioned by conspecifics. Proportion of M. vimineum in polyculture, an estimate of M. vimineum’s average fitness relative to P. pumila, increased across time since invasion in one year and was neutral in the other. The increase in stabilizing forces for M. vimineum suggests that stable coexistence between the invasive and native species may become more likely, although this may be countered in some years by increases in invader relative fitness.
Introduction

Despite intense competition for the same limiting resources, most plant communities are highly diverse. In the few instances where the strength of coexistence has been rigorously measured, plant communities appear to be strongly stabilized due to niche divergence among species (Levine and HilleRisLambers 2009, Adler et al. 2010, Chu and Adler 2015). Species invasions provide a counterexample to this phenomenon, since invasive species are often superior competitors compared to native species (Vila and Weiner 2004) and are associated with local declines in biodiversity and abundance of resident plant species in the invaded range (Vila et al. 2011). Invasive species’ ability to competitively exclude native plants could be due to their lack of coevolutionary history within the invaded range (Hallett 2006). For example, a release from a suppressive soil microbe community (Reinhart et al. 2003, Maron et al. 2014) could increase the invader’s ability to outcompete natives while also reducing the self-limitation that prevents native populations from reaching high densities. However, it is unclear how long this invasive species advantage, through evolutionary novelty, persists through invasion time (Lankau et al. 2009, Iacarella et al. 2015). An accumulation of a more antagonistic soil microbe community, or other factors, that limit the invader (Diez et al. 2010, Dostal et al. 2013) could shift competitive exclusion by an invader to coexistence between it and native species.

Whether species coexist or competitive exclusion occurs is determined by two factors, average fitness differences and stabilizing niche differences (Chesson 2000, Adler et al. 2007). Average fitness differences are those differences between species that support competitive exclusion (Chesson 2000, Adler et al. 2007), resulting in higher survival, fecundity, and/or growth of one species over another independent of relative abundance. Stabilizing niche differences are those differences that support coexistence by limiting conspecifics more strongly.
than heterospecifics (Chesson 2000, Adler et al. 2007). Stabilizing niche differences are represented by negative frequency dependence, i.e., decreasing per capita population growth rate with increasing frequency of conspecifics. Negative frequency dependence promotes coexistence by discouraging species extinction, through higher population growth rates when the species becomes rare, and reducing the potential of competitive exclusion, through lower population growth rates when the species becomes abundant in a community.

Coexistence could vary through invasion history, shifting from competitive exclusion by the invader to coexistence between it and native species, due to changes in both fitness differences and stabilizing niche differences. When an invasion is young and the invader is novel within the community it may have both higher fitness and reduced negative frequency dependence, possibly due to a less negative plant-soil microbe interaction since soil microbial communities can have strong population regulatory effects (Klironomos 2002, Mack and Bever 2014). However, plant-soil microbe interactions may become more negative for an invader over time potentially due to an accumulation of pathogens (Hawkes 2007, Diez et al. 2010, Mitchell et al. 2010, Dostal et al. 2013), decreasing the invader’s average fitness relative to native plants, while also increasing negative frequency dependence if the soil microbe community has frequency dependent effects (Kulmatiski et al. 2008). An increase in the invader’s negative frequency dependence and/or a decrease in its average fitness would make it more likely that native species could coexist.

We explored whether the components of coexistence between invasive Microstegium vimineum and native Pilea pumila varied across invasion history, and whether this pattern depended on the legacy of the invader on soils. These herbaceous species both have an annual life cycle, allowing for a more direct link between individual performance and population
dynamics, and have overlapping ranges and similar habitat preferences in the eastern United States. *Microstegium vimineum* has invaded a wide area in eastern North America via natural dispersal rather than intentional planting, and has multiple negative impacts on native flora, including reducing native plant biomass and diversity (Flory and Clay 2010a) and suppressing tree seedling regeneration (Flory and Clay 2010b). A prior observational study supports a decline in survival across invasion history for *M. vimineum* that is coupled with changes in its root/soil fungal community (chapter II), suggesting that an accumulation of a suppressive soil community may reduce the population growth and viability over invasion history.

We performed a two year field experiment where we estimated competitive ability at equal frequency (as a proxy for fitness differences) and negative frequency dependence (as a proxy for stabilizing niche differences) for *M. vimineum* and *P. pumila* across an invasion history gradient. Based on the hypothesis that *M. vimineum* has accumulated a more antagonistic soil microbial community over time, we made the following predictions: 1) *M. vimineum*’s negative frequency dependence would increase in invaded soil and would remain weak in uninvaded soil across invasion history, 2) there would be no trend in frequency dependence in either soil type across invasion history for *P. pumila*, since we have no reason to expect an increasing or decreasing negative/positive effect from the soil microbe community across sites for this native species, and 3) *M. vimineum*’s average fitness would be higher than *P. pumila*’s average fitness across invasion history in uninvaded soil, but would decrease relative to *P. pumila*’s average fitness across invasion history in invaded soil.

Additionally, we explored how short term soil conditioning by invasive *M. vimineum* or native *P. pumila* compared to the long-term conditioning inherent in invaded versus uninvaded soil across invasion history. We hypothesized that short term conditioning by *M. vimineum*
would maintain the buildup of a suppressive soil microbe community, especially in sites with a longer history of invasion, while conditioning by \textit{P. pumila} would decrease \textit{M. vimineum} specific pathogens in the soil. Based on that hypothesis, we expected \textit{M. vimineum}’s negative frequency dependence to increase, and its average fitness relative to native \textit{P. pumila} to decrease, in invaded soil across invasion history, and that this pattern would be maintained when conditioned, in the short term, by \textit{M. vimineum} and weakened when conditioned by \textit{P. pumila}.

\textbf{Methods}

\textbf{Study System}

\textit{Microstegium vimineum} is a C4, Asian annual grass. It was first collected within its invasive range in the United States in Knoxville, TN in 1919 (Fairbrot.De and Gray 1972). It is a shade tolerant invader in eastern forests (Winter et al. 1982). Multiple mechanisms have been proposed for \textit{M. vimineum}’s invasion success, including evolution post invasion (Flory et al. 2011), alteration in nutrient cycling (Ehrenfeld et al. 2001), and disturbance (Glasgow and Matlack 2007, Kuebbing et al. 2013).

\textbf{Year One: 2013}

To explore changes in coexistence of \textit{M. vimineum} and \textit{P. pumila} across invasion history we conducted a field experiment at sites that varied in estimated time since invasion by \textit{M. vimineum}. We chose 7 sites across the invasive range of \textit{M. vimineum} in the eastern United States that varied in estimated time since invasion, ranging from 11 to 47 years since invaded (Fig. 3.1). The estimated time since invasion of each site was determined from a map of \textit{M. vimineum} invasion history (Fig. 3.1, see chapter II for map details).

At each of the seven sites, we established a field experiment using a randomized complete block design with 5 blocks, a competition treatment (3 levels), and a soil treatment (2
levels) (Fig. 3.2). All blocks were set up in a forested area invaded by *M. vimineum* and were placed as close as possible to each other, but this was determined by where digging in the understory was possible.

Soil was collected throughout the invaded area of each site. We collected paired invaded and uninvaded soil from five different locations within each site. We collected invaded soil from highly invaded areas (> 75% cover of *M. vimineum*) and collected the paired uninvaded soil from an area without actively growing *M. vimineum* plants 1-3m away. Soil cores were dug to a depth of ~40 cm and the top ~15 cm was kept intact structurally, as best as possible, to try to reduce disturbance. Three invaded and three uninvaded soil cores were dug from each of the five different locations at each site. These six soil cores were used in the same block, therefore each of the five different soil collection locations were represented by one block. For each block we designated six 0.5m$^2$ units with flagging and randomly assigned each unit to a soil by competition treatment. We dug five gallon plastic grow bags with their bottoms removed into the ground and the soil cores were inserted into them, level with ground height. We maintained the top 15 cm of the soil core as the top soil in the unit. We removed plants growing in the top soil of the soil cores with scissors once we installed the cores into the blocks.

We had three competition treatments including monocultures of *M. vimineum* and *P. pumila*, and a polyculture of the two species combined. Each competition treatment contained nine individual plants. Polycultures had three individuals of both *M. vimineum* and *P. pumila*. Three individuals of a third species, native *Elymus virginicus*, were also planted into the polyculture cores, but this species had very low survival and so we removed it from all analyses. We germinated the plants in the greenhouse one month prior to field transplanting in circular cell pack tray inserts. Each circular cell was approximately 2 cm in diameter and 3 cm deep, and
filled with sterile potting soil. *Microstegium vimineum* seeds were collected from 9 sites, and *P. pumila* seeds from 2 sites across the eastern United States. For each competition treatment we made sure that each species’ seedlings came from a variety of sites and that this variety was kept consistent across treatments and blocks within sites as well as across sites in our field experiment. For *M. vimineum* we used all 9 maternal sites in our monocultures and 3 of these sites for polycultures and for *P. pumila* we used the same 2 maternal sites for all monocultures and polycultures.

We transported the seedlings out to the field and transplanted the complete plug directly into the soil cores. Each seedling was individually tagged to keep track of maternal site identity. We installed the experiment at each site from mid to late May 2013, and then two weeks later went back to replace any seedlings that had died and removed any non-experimental plants that sprouted in the units. To the best of our ability, we planted replacement seedlings from the same maternal site as the original seedlings. The plants grew through the season and then we collected each unit’s aboveground biomass, keeping every individual plant separate, from late September to early October 2013. We dried all plant material at 60°C and weighed their final biomass.

**Year Two: 2014**

In year two, 2014, we had a slightly different design then 2013 since the invaded and unininvaded soil was conditioned by either a polyculture, a *M. vimineum* monoculture, or a *P. pumila* monoculture. We will therefore refer to the soil type of each replicate soil core as either invaded or uninvaded, based on whether it was collected underneath or away from naturally occurring *M. vimineum* in 2013, and the conditioning treatment of each soil core as *M. vimineum, P. pumila*, or polyculture conditioned, based on the plant community grown in that soil core during the 2013 experiment. In 2014, we maintained the three competition treatments,
polyculture of *M. vimineum* and *P. pumila* and a monoculture of each species (Fig. 2). Monoculture plots contained ten individual plants of a single species and polycultures had five plants of each species. Methods for starting seedlings were the same as 2013, and once again we made sure that each species seedlings came from a variety of sites and that this variety was kept consistent across treatments and blocks within sites as well as across sites in our field experiment. For *M. vimineum* we had 5 maternal sites and we used all 5 for polycultures and monocultures. For *P. pumila* we had 4 maternal sites that were used in polycultures and monocultures.

In late May to early June 2014, we transplanted seedlings into the already established field units. Each seedling had an individual tag for maternal site identification. We planted polycultures into the plots that were conditioned, the year before, by monocultures of either *M. vimineum* or *P. pumila*. We planted monocultures into the plots that were conditioned the year before by polycultures of both species (Fig. 3.2). Due to this design, the monocultures were not represented in every block and due to low germination success we had only two monocultures of *P. pumila* at each of the 7 sites. In total we had 20 polycultures, five in each of the four soil by conditioning treatments (*M. vimineum* or *P. pumila* conditioned invaded or uninvaded soil), eight monocultures of *M. vimineum* (either in invaded or uninvaded, polyculture conditioned soil), and two monocultures of *P. pumila* (either in invaded or uninvaded, polyculture conditioned soil) at each site. We came back to each site two weeks after planting to replace any plants that had died, attempting to maintain the same maternal site identity for the replacement as the original plant. We allowed the plants to grow through the season and then, during late September and early October 2014, collected all of the aboveground biomass in each unit, keeping individual plants separate. We once again dried and weighed the plants for a final biomass.
In 2014, we measured other factors at each site that could be affecting fitness differences and negative frequency dependence within our experiment. During the replacement planting, we took canopy photos by holding a digital camera (Canon EOS Rebel T3) with a fish eye lens (Opteka) 1m above the center of each block at each site. We analyzed the photos for percent canopy openness using Gap Light Analyzer software (Frazer 1999). We also collected invaded and uninverted soil at each site to have a general nutrient analysis performed by the University of Georgia Soil, Water, and Plant Analysis Laboratory. Soil was collected near the five paired invaded/uninvaded soil collection areas from 2013. Invaded and uninverted soil collected from these five areas were combined to create one invaded and one uninverted soil sample per site. The test provided measurements of soil pH and extractable phosphorus, potassium, calcium, magnesium, manganese, and zinc. We also had a carbon and nitrogen test performed on the soil using Micro-Dumas combustion analysis at the Stable Isotope Ecology Laboratory at the University of Georgia. For these analyses we ball-milled the oven dried soil samples to less than 250 µm particle size and weighed 24-26 mg into 5 x 5 mm tin capsules. Through this test we received measures of soil total percent nitrogen and carbon and the C:N ratio.

To reduce the number of soil variables for analyses we performed a principal components analysis (PCA) in R version 3.2.2 using the 10 soil variables. We extracted the PCA scores for PCA axes 1, 2, and 3 and used these in future analyses. Axes 1, 2, and 3, combined, explained 94.5% of the variation in the soil variables. We had PCA scores for invaded and uninvaded soil separately for each site.

Before calculating unit averages for *M. vimineum* and *P. pumila* biomass we tested whether maternal site had an effect on individual biomass using linear mixed models. Maternal
site had a minimal effect on individual biomass, so we proceeded with averaging species biomass for every unit.

Relative Interaction Index

We calculated an overall site Relative Interaction Index (RII) (Armas et al. 2004) separately for *M. vimineum* and *P. pumila* in invaded and uninvaded soil in 2013 and for both species in invaded and uninvaded soil either conditioned by *M. vimineum* or *P. pumila* in 2014. Therefore we had 14 RII values in 2013 and 28 RII values in 2014 for *M. vimineum*. For *P. pumila* we had 14 RII values in 2013 and 16 RII values (ten from invaded soil and six from uninvaded soil) in 2014, because we did not have monocultures in both invaded and uninvaded soil at each site.

RII was calculated by subtracting polyculture average individual biomass from monoculture average individual biomass and then dividing by the sum of the polyculture and monoculture average individual biomass. When calculating monoculture average individual biomass, we excluded any individual plants whose maternal site was not also represented in the polyculture treatments. For the 2013 data, we first calculated the average individual biomass across the blocks at each site for polycultures and monocultures in invaded and uninvaded soil separately, and then used these values to calculate RII. We performed a similar calculation for the 2014 data except we used the average individual biomass for polycultures in *M. vimineum* or *P. pumila* conditioned invaded or uninvaded soil and monocultures in polyculture conditioned invaded or uninvaded soil to calculate RII. In 2014, *P. pumila* did not have monocultures in both polyculture conditioned invaded and uninvaded soil, so at most sites RII was only calculated for one of these soil types (except for HOF).
Monocultures represented high frequency plots and polycultures represented low frequency plots for each of our species. An RII > 0 indicates higher average individual biomass in monocultures compared to polycultures, suggesting positive frequency dependence. An RII < 0 indicates lower average individual biomass in monocultures compared to polycultures, suggesting negative frequency dependence.

Statistics

Changes in RII across time since invasion

To test for an increase in negative frequency dependence across time since invasion for *M. vimineum* we regressed RII against the estimated time since invasion at our 7 sites, and tested whether this relationship was affected by soil type (invaded/uninvaded) or the conditioning treatment (no additional conditioning for 2013 data or conditioned by *M. vimineum* or *P. pumila* for 2014 data). We ran a linear model that included a 3-way interaction (time since invasion * soil type * conditioning treatment) and percent canopy openness as a covariate. Because we hypothesized that relationships with time since invasion would be stronger in invaded soils, we then divided the data by soil type and ran a linear model within each soil type that included a 2-way interaction of time since invasion * conditioning treatment and included percent canopy openness as a covariate. In the 2013 data, site WF had an extreme value for RII (-1), since all *M. vimineum* plants in monoculture died at this site. Because of this, we also reran all the above analyses after removing site WF 2013 data points.

Since we did not have soil type represented across all sites for *P. pumila* in 2014, we did not include a 3-way interaction between time since invasion, soil type, and conditioning treatment in our linear models for *P. pumila* RII. Instead, to explore whether RII was changing across time since invasion for *P. pumila* we ran a linear model that included a 2-way interaction
between time since invasion and soil type, a 2-way interaction between time since invasion and conditioning treatment, a 2-way interaction between soil type and conditioning treatment, and percent canopy openness as a covariate. We then divided the data by soil type and ran a linear model within each type that included a 2-way interaction of time since invasion * conditioning treatment and included percent canopy openness as a covariate.

Changes in species proportional abundance across time since invasion

To explore whether there was evidence for a change in average fitness differences between *M. vimineum* and *P. pumila* across time since invasion we calculated the average proportion of total biomass in polyculture plots for *M. vimineum* and *P. pumila* across time since invasion separately for 2013 and 2014 invaded/uninvaded soil and the conditioning treatments. We regressed the proportional biomass of *M. vimineum* in polyculture against time since invasion and tested whether this relationship was affected by soil type or conditioning treatment. We included a 3-way interaction between time since invasion, soil type, and conditioning treatment and included percent canopy openness as a covariate. We then divided the data by soil type and ran a linear model with a 2-way interaction of time since invasion * conditioning treatment and included percent canopy openness as a covariate within each model.

We calculated the average total biomass of polycultures separately for 2013 and 2014 invaded/uninvaded soil and the conditioning treatments. Since average total biomass was not normally distributed and varied by several orders of magnitude between sites and years, we log transformed it prior to analyses. Using a linear model we tested whether the proportional biomass of *M. vimineum* in polyculture changed across time since invasion and whether this was affected by soil type and average total biomass. We included a 3-way interaction between time since invasion, soil type, and average total biomass and included percent canopy openness as a
covariate. We reran this analysis after removing site WF 2013, since this data appeared to be an outlier.

*Other factors potentially affecting RII and polyculture proportional abundance*

We ran linear models to test whether RII and/or *M. vimineum* proportional biomass in polyculture correlated with latitude or axes 1, 2, and 3 of our soil nutrient PCA. We also ran linear models to test whether latitude or the soil nutrient PCA axes were significantly correlated to time since invasion. Any factor significantly correlated to RII or *M. vimineum* proportional biomass was included in an additional linear model including the 3-way interaction of time since invasion, soil type, and conditioning treatment and either RII or *M. vimineum* proportional biomass as the dependent variable. Factors significantly correlated to RII were also included in a linear model with the 2-way interaction of time since invasion and conditioning treatment, using the data that was divided based on soil type.

**Results**

RII decreased across time since invasion for *M. vimineum* (Fig. 3.3), as evidenced by a significant main effect for time since invasion in the model containing the 3-way interaction between time since invasion, soil type, and conditioning treatment (F=7.6304, p=0.0099, Table 3.1). The decrease in RII across time since invasion was weaker in 2014 after the soil was conditioned by *P. pumila* (Fig. 3.3). This was shown by a marginally significant 2-way interaction between time since invasion and conditioning treatment (F=2.7628, p=0.0797, Table 3.1) in the larger model that included the 3-way interaction (time since invasion * soil type * conditioning treatment). RII tended to be higher in 2014 vs. 2013 (Fig. 3.3), as shown by a marginally significant main effect for conditioning treatment in the larger model (F=2.9651, p=0.0674, Table 3.1), although this may stem from methodological differences. When we
removed site WF 2013 from the dataset, time since invasion was no longer significant in this larger model (F=2.5690, p=0.1206, Fig 3.4)

RII decreased across time since invasion for *M. vimineum* in invaded soil, but not in uninvaded soil (Fig. 3.3), shown by a significant main effect for time since invasion in the model containing the 2-way interaction between time since invasion and conditioning treatment for invaded soils (F =8.3067, p=0.0121, Table 3.2). This pattern was maintained when we removed site WF 2013 from the dataset (F=5.5328, p=0.0351, Fig. 3.4). Axis 2 from the soil nutrient PCA was significantly negatively correlated with *M. vimineum*’s RII (F=5.1885, p=0.0282), while latitude and axes 1 and 3 from the soil nutrient PCA were not. PCA axis 2 explained 27.8% of the variation in the soil variables and the four nutrients that had the highest loadings on this axis were manganese, phosphorus, nitrogen, and carbon with loadings of -0.4644, 0.4158, -0.4836, and -0.5195 respectively. When axis 2 from the soil nutrient PCA was included in a model for RII with a 3-way interaction between time since invasion, soil type, and conditioning treatment, the main effect for time since invasion no longer had a significant effect. When the data was divided by soil type and axis 2 from the PCA was included in the model with a 2-way interaction of time since invasion and conditioning treatment, time since invasion maintained its significant correlation with RII in invaded soil (F=4.4263, p=0.0540). Time since invasion and axis 2 were not significantly correlated to RII in uninvaded soil and axis 2 was not correlated to RII in invaded soil.

There was a trend of RII decreasing across time since invasion for *P. pumila* (Fig. 3.5). This was shown by a marginally significant main effect of time since invasion (F=3.4225, p=0.0799, Table 3.1) in the larger model that included 2-way interactions between time since invasion and conditioning treatment, time since invasion and soil type, and soil type and
conditioning treatment. When we divided the data by soil type, RII decreased across time since invasion for *P. pumila* in invaded soil, but not in uninvaded soil (Fig. 3.5), shown by a significant main effect for time since invasion in the model containing the 2-way interaction between time since invasion and conditioning treatment for invaded soils (F=6.9336, p=0.0250, Table 3.2). The negative effect of time since invasion on RII in invaded soil is stronger when conditioned by either *M. vimineum* or *P. pumila* in 2014 (Fig. 3.5), shown by a slightly significant interaction between time since invasion and conditioning treatment (F=2.9429, p=0.0989, Table 3.2).

*Microstegium vimineum*’s proportion of total polyculture biomass tended to increase across time since invasion (F=2.9467, p=0.0967, Table 3.3, Fig. 3.6). The increase in *M. vimineum*’s proportion of total polyculture biomass became significantly stronger in 2014 regardless of whether the soil was conditioned by *M. vimineum* or *P. pumila* (Fig. 3.6), as evidenced by a significant 2-way interaction between time since invasion and conditioning treatment (F=4.7055, p=0.0170, Table 3.3). *Microstegium vimineum*’s proportion of total polyculture biomass was higher overall in 2014 compared to 2013 (Fig 3.6), shown by a significant main effect for conditioning treatment (F=9.8729, p=0.0005, Table 3.3). In invaded soil, the positive effect of time since invasion became stronger in 2014 regardless of whether it was conditioned by *M. vimineum* or *P. pumila* (Fig 3.6), shown by a marginally significant 2-way interaction between time since invasion and conditioning treatment (F=2.8764, p=0.0898).

The positive effect of time since invasion on *M. vimineum* proportion of total polyculture biomass became weaker at higher average total polyculture biomass (Fig 3.7), shown by a marginally significant interaction between time since invasion and average total polyculture biomass (F=3.0672, p=0.0892) in the model with the 3-way interaction between time since
invasion, soil type, and average total polyculture biomass. Average total polyculture biomass had an overall negative effect on *M. vimineum* proportion of polyculture biomass (F=3.6624, p=0.0644). After removing site WF 2013 from the dataset the interaction between time since invasion and average total polyculture biomass became stronger (F=5.9625, p=0.0205), the positive effect of time since invasion became significant (F=7.1433, p=0.0119), and the negative effect of average total polyculture biomass became stronger (F=7.4121, p=0.0105). Latitude and axes 1, 2, and 3 from the soil nutrient PCA were not correlated with *M. vimineum*’s proportion of total polyculture biomass.

**Discussion**

The advantage invasive species gain through their evolutionary novelty in their new communities could degrade through invasion time (Lankau et al. 2009, Iacarella et al. 2015), potentially due to an accumulation of a more suppressive soil community (Diez et al. 2010, Dostal et al. 2013). If this advantage degrades through time then competitive exclusion by the invader could shift to coexistence between it and native species, through a decline in the invader’s average fitness relative to natives and/or an increase in its negative frequency dependence. We explored whether invasive *M. vimineum*’s negative frequency dependence increased and average fitness declined relative to native *P. pumila*’s across time since invasion. Our data supports the former, but not the latter, suggesting that changes in average fitness across time since invasion are sensitive to year to year variation in productivity.

Negative frequency dependence for invasive *M. vimineum* increased across the gradient in time since invasion represented by our seven sites. When comparing patterns in invaded versus uninvaded soil, we found the effect of time since invasion was primarily in invaded soil, with more neutral patterns in uninvaded soil. This result is consistent with our original
hypothesis that soil conditioning by *M. vimineum* accumulates an antagonistic soil community to a greater degree in sites with longer invasion history, resulting in increased negative frequency dependence at long-invaded sites. We predicted that conditioning invaded soil with native *P. pumila* would weaken the build-up of negative frequency dependence across time since invasion, since this conditioning could reduce the amount of *M. vimineum* specific enemies in the soil. On the other hand, we predicted that conditioning with *M. vimineum* would maintain the *M. vimineum* specific pests and pathogens in the soil therefore maintaining the negative relationship between frequency dependence and time since invasion. Consistent with our hypothesis, in the invaded soil the conditioning treatments in 2014 shifted the relationship between RII and time since invasion, where conditioning by *P. pumila* weakened the negative relationship between RII and time since invasion while conditioning by *M. vimineum* maintained the relationship.

The increase in negative frequency dependence for *M. vimineum* across time since invasion, found to be more evident in invaded soil versus uninvaded soil, and maintained in *M. vimineum* conditioned soil and weakened in *P. pumila* soil, leads us to believe that the mechanism behind the pattern is soil driven and *M. vimineum* specific. Pathogen accumulation across time since introduction has been found for multiple non-native/invasive species (Hawkes 2007, Mitchell et al. 2010) and our data supports this as a possible mechanism for the increase in negative frequency dependence for *M. vimineum*. Pathogens, like many natural enemies, can be strong stabilizing forces for populations through negative frequency dependent effects (Chesson 2000, Mordecai 2011), since host-specific microbial populations can experience rapid, exponential growth when hosts are dense, leading to epidemics (Burdon and Chilvers 1982). We suspect that there is an accumulation of pathogens and/or other antagonists in *M. vimineum*’s soil community that increases stabilizing forces across invasion history. Soil feedbacks have been
shown to have large effects on community composition and dynamics, with a positive correlation between feedback response and relative abundance within a community (Klironomos 2002, Mangan et al. 2010). *Microstegium vimineum*’s increase in negative frequency dependence in invaded soil at sites with longer history of invasion suggests that its relative abundance will decline and stabilize over time due to population regulation by soil microbes.

Negative frequency dependence did not increase consistently across time since invasion for *P. pumila*, although there was a trend in this direction. The increase in negative frequency dependence across time since invasion for *P. pumila* was only evident in invaded soil in 2014, independent of whether the soil was conditioned by *M. vimineum* or *P. pumila*. However, we are missing data from two sites in this particular subset, leaving us with too few sites to make strong conclusions. In 2013, with the complete dataset, we found no relationship between frequency dependence and time since invasion for *P. pumila*, which is consistent with our hypotheses since we had no expectations for an increase or decrease in suppressive soil biota across time since *M. vimineum* invasion for this native species.

Our study suggests that *M. vimineum*’s fitness, relative to *P. pumila*, is variable across time since invasion. In 2013, we found no trend in the proportion of *M. vimineum* in polyculture across time since invasion. In 2014, relative to *P. pumila*, *M. vimineum*’s proportion of polyculture biomass increased across time since invasion, regardless of soil type or conditioning treatment. The 2014 results directly contradict our prediction that the proportion of *M. vimineum* in polyculture, a proxy for *M. vimineum*’s fitness relative to *P. pumila*, would decrease across time since invasion, potentially due to an accumulation of *M. vimineum* specific suppressive soil biota. The shift in the relationship between proportion of *M. vimineum* in polyculture and time since invasion from 2013 to 2014 could be partially explained by the yearly variation in total
productivity at each site. At younger invaded sites changes in the proportion of *M. vimineum* in polyculture was fairly constant across varying levels of productivity. However, at older invaded sites the proportion of *M. vimineum* in polyculture was negatively correlated to total productivity, with *M. vimineum* dominating in overall poor conditions, but subordinate to *P. pumila* in sites/years of high total plant growth. This pattern could relate to an accumulation of *M. vimineum* specific pathogens, since pathogen infection severity is known to fluctuate due to environmental variation (Scherm and Yang 1995, Prevey and Seastedt 2015). If good conditions for plant growth are also good for pathogen populations, *M. vimineum* growth in these years may be inhibited by large populations of pathogens that have accumulated at older invaded sites. Alternatively, if poor conditions limit pathogen populations as well as plant growth, then *M. vimineum* may be released from these specific pathogens and regain some of its invasive advantage. However, all of the trends for the proportion of *M. vimineum* in polyculture have been found in both invaded and uninvaded soil, reducing our confidence that these patterns arise solely through soil based mechanisms. There could be multiple mechanisms besides pathogens that are behind the shifting pattern of the proportion of *M. vimineum* in polyculture, including inherent differences among sites that pre-date the *M. vimineum* invasion.

The only environmental variable that was associated with changes in *M. vimineum*’s RII or proportion of *M. vimineum* in polyculture was PCA axis 2 from the soil nutrient PCA. PCA axis 2 was associated with changes in *M. vimineum*’s RII, but this did not conflict with the relationship between RII and time since invasion in invaded soil. Soil nutrients could potentially be a factor affecting frequency dependence, if intraspecific competition for the specific nutrients is stronger than interspecific competition due to resource partitioning (Chesson 2000, HilleRisLambers et al. 2012). Negative frequency dependence may increase as nutrients decrease
since lower amounts of nutrients in the soil would lead to increased competition over these resources, especially at higher conspecific frequency.

Increasing negative frequency dependence for *M. vimineum* across time since invasion suggests that stabilizing forces are accumulating. An increase in stabilizing forces will promote stable coexistence between *M. vimineum* and native plant species, and through time invaded communities may eventually resemble other natural communities with strong stabilizing forces (Levine and HilleRisLambers 2009, Adler et al. 2010, Chu and Adler 2015). However, while stabilizing forces are increasing over time, fitness differences for *M. vimineum* were variable from year to year at sites with longer invasion history. Increased fitness of *M. vimineum* relative to native species at older invaded sites could reduce the likelihood of coexistence even if stabilizing forces are accumulating.

Unfortunately, we were only able to estimate proxies for relative fitness differences and stabilizing forces, rather than obtaining more accurate values by incorporating population vital rate measurements. Future work should more accurately assess these parameters. Also we only used one native species in our study, but *M. vimineum* will be competing with multiple native species across its invasive range. Estimates of fitness differences and negative frequency dependence, and thus conclusions regarding long-term coexistence or competitive exclusion, could vary depending on the specific species with which *M. vimineum* is competing. However, the general trend of stabilizing forces accumulating would likely remain the same, as this phenomenon was specific to *M. vimineum* and its interaction with its conspecific conditioned soils. Thus, while we cannot state conclusively whether *M. vimineum* and *P. pumila* will coexist over the long-term, from this study we can conclude that the likelihood of coexistence is
increasing over time due to an accumulation of population stabilizing forces for invasive *M. vimineum*.

**References**


Chu, C. J., and P. B. Adler. 2015. Large niche differences emerge at the recruitment stage to stabilize grassland coexistence. Ecological Monographs **85**:373-392.


Frazer, G. W., C. D. Canham, and K. P. Lertzman. 1999. Gap Light Analyzer (GLA), version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia and the Institute of Ecosystem Studies, Millbrook, NY.


Table 3.1 - Results of linear model for each species with the relative interaction index (RII) as the dependent variable. Testing whether RII is associated with time since *Microstegium vimineum* invasion (years), soil type (invaded or uninvaded), or conditioning treatment (unconditioned 2013 data, *M. vimineum* conditioned 2014 data, *P. pumila* conditioned 2014 data), their interactions, and using % canopy openness as a covariate. R² is for the overall model fit.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model term</th>
<th>F value</th>
<th>p value</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time since invasion</td>
<td>7.6304</td>
<td>0.0099</td>
<td><strong>0.4359</strong></td>
</tr>
<tr>
<td></td>
<td>soil type</td>
<td>0.4697</td>
<td>0.4986</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conditioning treatment</td>
<td>2.9651</td>
<td>0.0674</td>
<td>*</td>
</tr>
<tr>
<td>Microstegium vimineum</td>
<td>% canopy openness</td>
<td>4.2954</td>
<td>0.0472</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>time*soil</td>
<td>0.3210</td>
<td>0.5754</td>
<td></td>
</tr>
<tr>
<td></td>
<td>time*conditioning</td>
<td>2.7628</td>
<td>0.0797</td>
<td>*</td>
</tr>
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<td></td>
<td>soil*conditioning</td>
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<td>0.8212</td>
<td></td>
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<tr>
<td></td>
<td>time<em>soil</em>conditioning</td>
<td>0.4397</td>
<td>0.6484</td>
<td></td>
</tr>
<tr>
<td>Pilea pumila</td>
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<td>0.0799</td>
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<td></td>
<td>soil type</td>
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<td>0.5549</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conditioning treatment</td>
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<td>0.5474</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% canopy openness</td>
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<td>time*soil</td>
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<td>0.7204</td>
<td></td>
</tr>
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<td></td>
<td>time*conditioning</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>soil*conditioning</td>
<td>0.5007</td>
<td>0.6139</td>
<td></td>
</tr>
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</table>
### Table 3.2

Results of linear model for each species and soil type with the relative interaction index (RII) as the dependent variable.

Testing whether RII is associated with time since Microstegium vimineum invasion (years), conditioning treatment (unconditioned 2013 data, M. vimineum conditioned 2014 data, P. pumila conditioned 2014 data), their interaction, and using % canopy openness as a covariate. $R^2$ is for the overall model fit.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model term</th>
<th>P value</th>
<th>F value</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invaded soil</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microstegium</td>
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<tr>
<td>0.2384</td>
<td>0.1878</td>
<td>*</td>
<td>0.0699</td>
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<tr>
<td>0.1981</td>
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<td>**</td>
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<td>0.5733</td>
<td></td>
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<td>0.0235</td>
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<td>0.5221</td>
<td>0.6533</td>
<td></td>
<td>0.5733</td>
<td>0.0235</td>
</tr>
<tr>
<td>P. pumila</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1872</td>
<td>0.1187</td>
<td>**</td>
<td>0.0977</td>
<td>0.0121</td>
</tr>
<tr>
<td>0.3383</td>
<td>0.5938</td>
<td>**</td>
<td>0.0938</td>
<td>0.0121</td>
</tr>
<tr>
<td>0.3142</td>
<td>0.5938</td>
<td>**</td>
<td>0.0938</td>
<td>0.0121</td>
</tr>
<tr>
<td>0.3414</td>
<td>0.5938</td>
<td>**</td>
<td>0.0938</td>
<td>0.0121</td>
</tr>
</tbody>
</table>

Table 3.2 - Results of linear model for each species and soil type with the relative interaction index (RII) as the dependent variable.
Table 3.3- Results of linear model with *Microstegium vimineum*’s proportion of polyculture biomass as the dependent variable. Testing whether *M. vimineum*’s proportion of polyculture biomass is associated with time since *Microstegium vimineum* invasion (years), soil type (invaded or uninvaded), conditioning treatment (unconditioned 2013 data, *M. vimineum* conditioned 2014 data, *P. pumila* conditioned 2014 data), their interaction, and using % canopy openness as a covariate. $R^2$ is for the overall model fit.

<table>
<thead>
<tr>
<th>Model term</th>
<th>F value</th>
<th>p value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>time since invasion</td>
<td>2.9467</td>
<td>0.0967 *</td>
<td>0.5785</td>
</tr>
<tr>
<td>soil type</td>
<td>0.2692</td>
<td>0.6078</td>
<td></td>
</tr>
<tr>
<td>conditioning treatment</td>
<td>9.8729</td>
<td>0.0005 ***</td>
<td></td>
</tr>
<tr>
<td>% canopy openness</td>
<td>2.6302</td>
<td>0.1157</td>
<td></td>
</tr>
<tr>
<td>time*soil</td>
<td>0.0000</td>
<td>0.9961</td>
<td></td>
</tr>
<tr>
<td>time*conditioning</td>
<td>4.7055</td>
<td>0.0170 **</td>
<td></td>
</tr>
<tr>
<td>soil*conditioning</td>
<td>0.5234</td>
<td>0.5980</td>
<td></td>
</tr>
<tr>
<td>time<em>soil</em>conditioning</td>
<td>0.1991</td>
<td>0.8206</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1 - Map of *Microstegium vimineum* invasion history, created using the kriging function in ArcMap from ArcGIS 10.1. Points on map are the 7 sites where the experiment was performed and are also listed in the table with their abbreviated identification, state of location, and estimated time since invasion (years).

<table>
<thead>
<tr>
<th>Site</th>
<th>State</th>
<th>Time since invasion (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>IN</td>
<td>11</td>
</tr>
<tr>
<td>HOF</td>
<td>NC</td>
<td>16</td>
</tr>
<tr>
<td>CHOC</td>
<td>AL</td>
<td>20</td>
</tr>
<tr>
<td>SC</td>
<td>MD</td>
<td>26</td>
</tr>
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<td>CNSC</td>
<td>VA</td>
<td>35</td>
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<tr>
<td>WF</td>
<td>GA</td>
<td>43</td>
</tr>
<tr>
<td>DF</td>
<td>NC</td>
<td>47</td>
</tr>
</tbody>
</table>
Figure 3.2 - Experimental design for the same block across seasons 1 and 2. There were 5 blocks at each site in a randomized complete block design for season 1. The soil treatment consisted of two soil types, invaded (yellow) and uninvaded (peach). In season 1, the 3 competition treatments (monocultures of *Microstegium vimineum* and *Pilea pumila* and polyculture of the two species mixed) were replicated across soil type and 9 individual plants were in each unit. In season 2 we repeated the 3 competition treatments (10 plants/unit) and replicated the polyculture in the 2 soil types, except now all units had a conditioning treatment from season 1, shown in parentheses: MV= *M. vimineum* monoculture, PP= *P. pumila* monoculture, PC= polyculture of both species. In season 2, polycultures were planted in MV or PP conditioned units across both soil types and monocultures were planted in PC conditioned units. Due to only having 1 PC conditioned unit per soil type in each block we were unable to have monocultures of each species replicated across both soil types in each block.
Figure 3.3- Microstegium vimineum’s relative interaction index (RII) across time since M. vimineum invasion (years). Panel A is in invaded soil, blue points are the 2013 unconditioned treatment, orange points are the 2014 M. vimineum conditioned treatment, and green points are the 2014 Pilea pumila conditioned treatment. Trend lines and R^2 values correspond to the conditioning treatment in the same color. Panel C is in uninvaded soil and has the same color code as A. Panel B is in invaded soil and fits a trend line through the combined conditioning treatment data after detrending the data for year and % canopy openness. Panel D is in uninvaded soil and has the same description as Panel B.
Figure 3.4- *Microstegium vimineum*’s relative interaction index (RII) across time since *M. vimineum* invasion (years) after removing the data point for site WF, season 1. Panel A is in invaded soil, blue points are the 2013 unconditioned treatment, orange points are the 2014 *M. vimineum* conditioned treatment, and green points are the 2014 *Pilea pumila* conditioned treatment. Trend lines and $R^2$ values correspond to the conditioning treatment in the same color. Panel C is in uninvaded soil and has the same color code as A. Panel B is in invaded soil and fits a trend line through the combined conditioning treatment data after detrending the data for year and % canopy openness. Panel D is in uninvaded soil and has the same description as Panel B.
Figure 3.5- *Pilea pumila*’s relative interaction index (RII) across time since *M. vimineum* invasion (years). Panel A is in invaded soil, blue points are the 2013 unconditioned treatment, orange points are the 2014 *M. vimineum* conditioned treatment, and green points are the 2014 *Pilea pumila* conditioned treatment. Trend lines and $R^2$ values correspond to the conditioning treatment in the same color. Panel C is in uninvaded soil and has the same color code as A. Panel B is in invaded soil and fits a trend line through the combined conditioning treatment data after detrending the data for year and % canopy openness. Panel D is in uninvaded soil and has the same description as Panel B.
Figure 3.6- *Microstegium vimineum*’s proportion of polyculture biomass across time since *M. vimineum* invasion (years). Panel A is in invaded soil, blue points are the 2013 unconditioned treatment, orange points are the 2014 *M. vimineum* conditioned treatment, and green points are the 2014 *Pilea pumila* conditioned treatment. Trend lines and $R^2$ values correspond to the conditioning treatment in the same color. Panel B is in uninvaded soil and has the same color code as A.
Figure 3.7- Contour plot of *Microstegium vimineum*’s proportion of polyculture biomass across time since *M. vimineum* invasion and the natural log of the average total polyculture biomass. Data was averaged for each soil type by conditioning treatment for each site varying in time since *M. vimineum* invasion.
CHAPTER IV:

SOIL BIOTA PROMOTE COEXISTENCE BETWEEN AN INVASIVE AND NATIVE PLANT

3 Cunard C and Lankau R. To be submitted to Ecology.
Abstract

Soil microbes may promote coexistence of plant species by increasing stabilizing niche differences, i.e. negative frequency dependence, and/or reducing average fitness differences between species. Many invasive species disrupt coexistence and become dominant in plant communities, potentially due to less negative interactions with soil microbes via enemy release. However, whether this less negative plant-soil biota interaction is maintained across invasion time is unclear; accumulation of pests and pathogens could promote coexistence between invasive and native species. Through a greenhouse experiment we explored whether soil biota increased the likelihood of coexistence between invasive *Microstegium vimineum* and native *Pilea pumila* and whether this effect increased across soil invasion history. We found that soil biota increased the likelihood of coexistence, but this was not increasing across soil invasion history. Soil biota increased the likelihood of coexistence through increased stabilizing niche differences rather than changes in average fitness differences, suggesting that soil biota play a strong role in plant-plant interactions, which are often overlooked in traditional plant-soil feedback experiments. Plant performance for both species generally increased across soil invasion history in sterile soil, but remained constantly low in whole soil, suggesting an accumulation of an antagonistic soil community that increasingly suppresses plant performance. To our knowledge, this is the first study to thoroughly assess the role of soil biota in maintaining coexistence between plant species using modern coexistence theory.
Introduction

In classical plant ecology theory, coexistence and the relative abundance of plant species within communities have been explained by resource competition. Species dominance or rarity is driven by their ability to acquire resources relative to other species and coexistence is stabilized when species differ in their resource requirements or the space and timing of their resource acquisition (Grime 1979, Tilman 1982). More recently, plant-soil feedbacks, driven by the soil microbial community, have been proposed as a mechanism for coexistence, due to their ability to drive negative frequency dependence within plant species (Bell et al. 2006, Bever et al. 2010, Bagchi et al. 2014) and their correlation to plant species’ relative abundances (Bever et al. 1997, Klironomos 2002, Kulmatiski et al. 2008, Mangan et al. 2010, MacDougall et al. 2011). However, negative frequency dependence is only one component of coexistence (Chesson 2000), and it remains unclear how the multifaceted effects of soil microbial communities throughout plant lifecycles will ultimately shape the long-term dynamics of interacting plant species.

Whether species coexist or one competitively excludes the other is determined by stabilizing niche differences and average fitness differences (Chesson 2000). Stabilizing niche differences are differences between species that promote coexistence by limiting conspecifics more strongly than heterospecifics (Chesson 2000, Adler et al. 2007). Stabilizing niche differences result in negative frequency dependence for a species, i.e. decreasing per capita population growth rate with increasing conspecific frequency (Chesson 2000). Negative frequency dependence promotes coexistence through a higher per capita population growth rate when a species is rare within a community, discouraging species extinction, and lower per capita population growth rate when a species is abundant relative to other species within a community, discouraging competitive exclusion. Average fitness differences are differences between species that promote
competitive exclusion and are overall differences in demographic vital rates (e.g. survival, fecundity, and growth) or response to competition that are independent of species relative abundances within the community (Chesson 2000).

Soil microbial communities may affect the two components of coexistence in several ways. For instance, pathogens can exert negative frequency dependence when disease transmission correlates with host density, leading to a higher probability of epidemics in dense populations (Burdon and Chilvers 1982), potentially stabilizing coexistence. Alternatively, access to soil mutualists may increase the average fitness of one plant species over another, reducing the likelihood of coexistence. Plant species can alter the assembly of soil microbial communities in their root zone, and these legacies provide another source of stabilizing or destabilizing effects. Negative plant soil feedbacks, where plant species condition microbial communities detrimental to conspecifics, may promote coexistence and plant diversity within a community both by lowering overall fitness and by creating negative frequency dependence, as germinants are more likely to encounter detrimental soils when their conspecifics are at high frequency (Kulmatiski et al. 2008). Positive plant soil feedbacks, where plant species condition communities beneficial to conspecifics, may promote the dominance of a species, or even destabilizing priority effects, by the creation of positive frequency dependence (Bever et al. 2010, van der Putten et al. 2013).

Coexistence between plant species, in the few cases that it has been thoroughly assessed, is strongly stabilized due to niche divergence (Levine and HilleRisLambers 2009, Adler et al. 2010, Chu and Adler 2015). However, these studies have taken a phenomenological approach to estimating coexistence, and thus the ecological mechanisms creating this strong stabilization are not clear (Levine and HilleRisLambers 2009, Adler et al. 2010, Chu and Adler 2015). While soil microbial communities have clear potential to stabilize coexistence, no studies to date have
experimentally manipulated soil microbial communities to determine their net effect on plant population dynamics and formal coexistence components.

Natural communities that are invaded by exotic species are associated with declines in resident plant species diversity and abundances (Vila et al. 2011) and are an interesting case in which coexistence appears to break down. Since the soil microbe community may promote coexistence through negative plant-soil feedbacks, invader dominance may arise due to a tendency for invaders to have neutral or positive plant-soil feedbacks (Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004b). A more positive plant-soil interaction in the invaded range could be due to an absence of specialized enemies or novel mutualistic interactions (Reinhart and Callaway 2006). However, it is unclear whether this positive plant-soil interaction, and thus competitive dominance, persists or degrades through invasion history to promote coexistence between the invasive and native plant species (Diez et al. 2010, Dostal et al. 2013, Day et al. 2015).

Plant-soil feedbacks for nonnative species have been shown to become more negative across time since establishment (Diez et al. 2010, Dostal et al. 2013). A soil microbe community with a short invasion history is potentially lacking the specialized pests and pathogens present in the native range of the invader (Reinhart and Callaway 2006). A more positive plant-microbe interaction could promote competitive exclusion by the invader through weak negative frequency dependence and high average fitness relative to native plants in the community. A soil microbe community with a long invasion history has potentially accumulated specialized pests and pathogens (Hawkes 2007, Mitchell et al. 2010, Schultheis et al. 2015) causing for a more negative plant-soil feedback (Diez et al. 2010, Dostal et al. 2013). A more negative plant-soil microbe interaction could promote coexistence between the invader and the native community.
through increased stabilizing forces, i.e. negative frequency dependence, and reduced average fitness.

In this study, we explored whether the likelihood of coexistence between invasive *Microstegium vimineum* and native *Pilea pumila* changed due to the presence and/or the invasion history of the soil microbial community. These two species are both annuals, have overlapping ranges in the eastern United States, and have similar habitat preferences. *Microstegium vimineum* is an aggressive grass invader that has spread throughout the eastern United States through natural dispersal rather than intentional planting. It has multiple negative effects on native plant species including reducing native plant biomass and diversity (Flory and Clay 2010a) and suppressing tree seedling regeneration (Flory and Clay 2010b). In a prior observational study and field experiment, *M. vimineum*’s frequency dependence in invaded soil as well as the soil/root fungal community changed across time since invasion, suggesting microbes may be promoting coexistence as invasion time increases (Cunard & Lankau, chapter II & III).

We performed a greenhouse experiment with a response surface design to address whether the propensity for coexistence or any of the underlying demographic parameters were affected by the presence and/or the invasion history of the soil microbial community. Based on the hypothesis that a *M. vimineum* specific antagonistic soil microbial community has accumulated across invasion history we expected that 1) the presence of the soil microbial community would increase the likelihood of coexistence overall, 2) the likelihood of coexistence would also increase across invasion history of the soil microbial community, and 3) *M. vimineum* specific demographic parameters would change across invasion history and the presence of the soil microbial community, but *P. pumila* specific parameters would only change due to the presence of the soil microbial community.
Methods

Study Species

*Microstegium vimineum* is an Asian, C4 annual grass. It was first collected in its invaded range in the United States in Knoxville, TN in 1919 (Fairbrot.De and Gray 1972). *Microstegium vimineum* is an aggressive shade tolerant invader of eastern US forests (Winter et al. 1982) and is commonly found in moist habitats such as floodplains, stream banks, and riparian areas (Redman 1995). The reasons for *M. vimineum*’s invasion success are not clear, but multiple mechanisms have been proposed including evolution post invasion (Flory et al. 2011), alteration in nutrient cycling (Ehrenfeld et al. 2001), and disturbance (Glasgow and Matlack 2007, Kuebbing et al. 2013).

Greenhouse Experiment

We performed a greenhouse experiment in which we competed *M. vimineum* and *P. pumila* in a response surface design across 7 soils that varied in *M. vimineum* invasion history and 2 soil biota treatments, soil kept whole or sterilized.

We collected *M. vimineum* invaded soil from 7 sites that varied in estimated time since invasion by *M. vimineum* (Fig. 4.1). Sites ranged from 11 to 47 years since invaded by *M. vimineum*. Time since invasion for each site was estimated from a map of *M. vimineum* invasion history (Fig. 4.1, see chapter II for map details). We collected soil from each site from late September to early October 2014. All areas where soil was collected had ≥ 75% cover of *M. vimineum* and we collected the top 15 cm of soil from three different locations and then pooled them for one soil sample per site. The soil was kept as cool as possible during field collections and then stored in a cold room at 4°C until the start of the experiment.
We steam sterilized half of the soil from each of the 7 sites twice, letting them cool in between each sterilization treatment. We collected a soil sample from the 14 treatment combinations (7 invasion histories X 2 soil treatments) to have a general soil nutrient analysis performed by the Georgia Soil, Water, and Plant Analysis Laboratory (Athens, GA) that provides measurements on soil pH and extractable phosphorous, potassium, calcium, magnesium, manganese, and zinc. We also received measures of total percent carbon and nitrogen through Micro-Dumas combustion analysis at the Stable Isotope Ecology Laboratory at the University of Georgia (Athens, GA).

We mixed each soil type with sand in a 2:1 ratio. We filled 1L rectangular pots with 0.75 L of the soil-sand mixture. Six pots were placed together in a tray, maintaining the same soil invasion history and biota treatment within each tray, except for one tray per soil invasion history had 2 sterilized soil units with 4 whole soil units. We filled all the pots and placed them out in a greenhouse for two weeks, where they were watered regularly. In this two week period we pulled out any plants that germinated in the soil treatments and this period was also used to leach nutrients that became available through the sterilization process. In total we had 448 pots, 64 for each of the 7 soils varying in *M. vimineum* invasion history with half of them (32 units) sterilized.

After two weeks, we set up a response surface design in each of the 14 treatment combinations, with varying densities of *M. vimineum* and *P. pumila* all in combination (Fig. 4.2). Densities of *M. vimineum* and *P. pumila* were 0, 1, 4, 7, and 10 individuals. Each combination of densities was represented once in each of the soil X invasion history treatments, except for the combination where one *M. vimineum* or *P. pumila* individual grew alone, which was replicated 5 times. To set up the response surface design we planted a set number of seeds of each species
into each pot. For every specified density of *M. vimineum/P. pumila* we planted 3 times the number of seeds, so for the densities of 0, 1, 4, 7, and 10 individuals we planted 0, 3, 12, 21, and 30 seeds.

Seeds of *M. vimineum* and *P. pumila* were collected at sites throughout the eastern United States from 2010 to 2013. We used *M. vimineum* and *P. pumila* seeds from 12 and 4 different sites, respectively, and from multiple maternal individuals at each site, ranging from 7 to 33 individuals. Prior experiments found no significant differences among populations in fitness across field sites and competition treatments (Cunard unpub. data). Therefore, we pooled all the seeds for each species. Once all of the units were planted we randomized the trays in the greenhouse.

Two and three weeks after we planted seeds, we counted the number of *M. vimineum* and *P. pumila* germinants in all of the units. Four weeks after we planted the seeds, we did a final germinant count and then pulled out or transplanted in any *M. vimineum* or *P. pumila* individuals necessary to establish the appropriate densities in each unit. We only transplanted individuals from units that had the same invasion history and soil treatment as the unit that was receiving the transplant. Two weeks after initiating the experiment with the appropriate densities of *M. vimineum* and *P. pumila* we replaced any individuals that had died using seedlings we had grown in trays of sterilized potting soil. Midway through the experiment, approximately 1.5 months, we re-randomized the trays.

We harvested the experiment after 3 months, when most plants reached maturity. We collected the aboveground biomass of all the units, keeping species separate. We dried all of the biomass at 60°C and then weighed it. We calculated individual biomass for each species for each unit by dividing the total biomass by the number of individuals in the specific unit. We estimated
individual plant seed production based on aboveground biomass, since biomass is a good predictor of seed production for both species (*M. vimineum*: $R^2=0.881$, $P<0.00001$, $n=27$; *P. pumila*: $R^2=0.8104$, $P<0.00001$, $n=25$).

**Modelling Coexistence**

We used equations from Godoy & Levine (2014) to calculate stabilizing niche differences and average fitness differences. Equations were derived using the following model that describes the population dynamics of an annual plant with a seedbank (Chesson 1990, Levine and HilleRisLambers 2009, Godoy and Levine 2014)

$$\frac{N_{i,t+1}}{N_{i,t}} = (1-g_i) s_i + g_i F_i$$

(1)

Where $N_{i,t}$ is the number of seeds in the soil before germination and the per capita population growth rate ($N_{i,t+1}/N_{i,t}$) is determined by the germination fraction ($g_i$), the annual survival of ungerminated seeds in the soil ($s_i$), and the per-germinant fecundity ($F_i$).

Godoy & Levine (2014) then expanded the per-germinant fecundity ($F_i$) into a function that takes into account the effect of competing individuals.

$$F_i = \frac{\lambda_i}{1 + \alpha_{ij} g_i N_{i,t} + \alpha_{ij} g_j N_{j,t}}$$

(2)

In which, $\lambda_i$ is the per-germinant fecundity in the absence of competition and $\alpha$ is an interaction coefficient, where $\alpha_{ij}$ is the per capita effect of species $j$ on species $i$.

Niche overlap ($\rho$) between species is

$$\rho = \sqrt{\frac{\alpha_{ij}}{\alpha_{jj}}} \frac{\alpha_{ji}}{\alpha_{ii}}$$

(3)
Niche overlap equal to 1 indicates that species equally limit conspecifics and heterospecifics, while a p of 0 indicates complete niche separation, in which species have no effect on heterospecifics. Niche overlap will be greater than 1 if species limit heterospecifics more strongly than they limit conspecifics. Stabilizing niche differences are calculated as $1 - \rho$, where positive values indicate stabilizing negative frequency dependence, while negative values indicate destabilizing positive frequency dependence.

The average fitness ratio ($\kappa_j / \kappa_i$) is

$$\frac{\kappa_j}{\kappa_i} = \left( \frac{\eta_j - 1}{\eta_i - 1} \right) \frac{\alpha_{ij}}{\alpha_{ji}} \frac{\alpha_{ij}}{\alpha_{ji}}$$

(4)

where the productivity ($\eta_i$), the annual seed production per seed loss from the seedbank, is

$$\eta_i = \frac{\lambda_i g_i}{1 - (1 - g_i)(s_i)}$$

(5)

If the average fitness ratio is greater than 1 species $j$ is the better competitor and would outcompete species $i$ if there was perfect niche overlap; the opposite is true if the ratio is less than 1. The average fitness ratio is comprised of a “demographic ratio”

$$\frac{\eta_j - 1}{\eta_i - 1}$$

(6)

and a “competitive response ratio”

$$\sqrt{\frac{\alpha_{ij}}{\alpha_{ji}} \frac{\alpha_{ij}}{\alpha_{ji}}}$$

(7)

The demographic ratio describes the degree to which species $j$ produces more seeds per seed loss due to death or germination than species $i$. The competitive response ratio describes the degree to which species $j$ is more resistant to competition than species $i$ (Godoy and Levine 2014).
Coexistence occurs when both species can invade a resident population of the other and occurs when

$$\rho < \frac{k_j}{k_i} < \frac{1}{\rho}$$  \hspace{1cm} (7)

Species $i$ can invade species $j$ when $\alpha'_{ji} > \alpha'_{ij}$ and species $j$ can invade species $i$ when $\alpha'_{ii} > \alpha'_{ji}$, where

$$\alpha'_{ij} = \frac{g_j a_{ij}}{\eta_i - 1}$$  \hspace{1cm} (8)

Parameterizing stabilizing niche differences and average fitness differences

We parameterized the above models for each of the soil by invasion history treatments using the greenhouse experiment. We calculated the germination fraction for *M. vimineum* and *P. pumila* by dividing the total number of germinants by the total number of seeds planted for each of the 14 treatment combinations. To account for germinants that grew from the seed bank rather than the experimental planted seed we averaged the number of germinants occurring in units that did not have any *M. vimineum* or *P. pumila* experimental seeds added, separately for each soil by invasion history treatment. We then subtracted this average from every units’ germinant count before calculating the total number of germinants for that specific treatment combination. We were unable to estimate annual seed survival in the soil ($s_i$). Therefore, we set this value to 0.5 for both species in all of our calculations.

We used maximum likelihood methods (function “optim”, estimation method “L-BFGS-B”, and lognormal error structure) in R version 3.2.2 to fit $\lambda$ and the interaction coefficients ($\alpha$) using the equation for per-germinant fecundity ($F_i$, Eq. 2) and the data collected on per-germinant fecundity at the varying densities of *M. vimineum* and *P. pumila* in the greenhouse experiment. To fit $\lambda$ and the interaction coefficients for *M. vimineum* we set the initial values for
sigma (variance parameter for the log-normal distribution) to 2, for the interaction coefficients to 1, and for λ to 300, which was approximately the average λ across all of our treatment combinations for *M. vimineum*. To fit λ and the interaction coefficients for *P. pumila* we set the starting point for sigma to 2, for the interactions coefficients to 1, and for λ to 275, which was approximately the average λ across all of our treatment combinations for *P. pumila*. We constrained all values to be positive. We used Eq. 2, except instead of using \( \frac{N_i}{N_j} \) and \( \frac{g_i}{g_j} \) we substituted the number of *M. vimineum* and *P. pumila* competitors in each pot, since we knew these exact densities from our experiment. We obtained estimates for *M. vimineum*’s and *P. pumila*’s λ and the four interaction coefficients for each soil by invasion history treatment.

Once we obtained estimates for all of the parameters we calculated niche overlap, stabilizing niche differences, and the average fitness ratio and determined whether *M. vimineum* and *P. pumila* were predicted to coexist in each of the soil by invasion history treatments. We created functions that depicted the barrier between competitive exclusion and coexistence. Since the average fitness ratio \( \frac{\kappa_j}{\kappa_i} \) has to be greater than niche overlap \( \rho \), but also less than \( 1/\rho \), to allow coexistence, we set the average fitness ratio equal to niche overlap for one function and set it equal to \( 1/\text{niche overlap} \) for the second function, constraining both functions to the region of niche overlap less than one. Then to calculate the likelihood of coexistence for each point we calculated the minimum distance between the stabilizing niche differences and average fitness ratios for each soil by invasion history combination to the two functions. This provided a quantitative measure of placement of each community in the coexistence-exclusion space. Communities determined to coexist where given a “distance” of 0.

We calculated \( \alpha' \) for each of the interaction combinations, using Eq. 8, to determine whether *M. vimineum* and/or *P. pumila* could invade when rare in each soil by invasion history...
treatment. We subtracted $\alpha'_{ji}$ and $\alpha'_{ij}$ from $\alpha'_{jj}$ and $\alpha'_{ii}$ respectively to obtain an estimate of how close the species is to being able to invade when rare. A positive value means the species is able to invade when rare, while a negative value means it will not.

To test whether any demographic parameters varied with the presence/absence of a soil community, and across the soil invasion history gradient, we used linear models that included a 2-way interaction between soil treatment and invasion history for each of the separate demographic parameters including *M. vimineum* and *P. pumila’s* germination rate ($g$), per-germinant fecundity in the absence of competition ($\lambda$), and productivity ($\eta$). We also ran this same linear model for average fitness differences, stabilizing niche differences, distance to coexistence, the four interaction coefficients ($\alpha$), the demographic ratio, and the competition ratio. We ran this linear model for both species’ ability to invade when rare as well by using the values obtained from subtracting $\alpha'_{ij}$ and $\alpha'_{ji}$ from $\alpha'_{jj}$ and $\alpha'_{ii}$ respectively.

We condensed the 9 soil nutrients variables using a principal components analysis (PCA) in R version 3.2.2. We used PCA axes 1, 2, and 3 in analyses. PCA axes 1, 2, and 3 explained 41.57%, 34.73%, and 14.82% of the total variation in the soil nutrient variables respectively, for a total of 91.13% of the variation explained. We regressed each of the three axes across invasion history, the soil treatment, and their interaction, to explore whether the nutrient variables were associated with invasion history and whether this was dependent on whether the soil was whole or sterile.

**Results**

*Microstegium vimineum* and *P. pumila* met the criteria for coexistence in two out of the fourteen soil by invasion history treatments (sites HOF and CNSC, both in whole soil) (Fig. 4.3A). The niche overlap ($\rho$) was 0.2814 and 0.3801, the average fitness ratio ($k_j/k_i$) was 0.9729
and 0.6536, and 1/\rho was 3.5542 and 2.6312 in the whole soil for sites HOF and CNSC, respectively. Distance to coexistence was significantly shorter with whole soil compared to sterile soil, as evidenced by a significant main effect for the soil treatment (F=5.9094, p=0.0354, Table 4.1, Fig 4.3B). Average fitness differences were not associated with the soil treatment, invasion history, or their interaction (Table 4.1, Fig 4.3D). Stabilizing niche differences were significantly larger in whole soil compared to sterile soil, shown by a significant main effect for the soil treatment (F=6.3321, p=0.0306, Table 4.1, Fig 4.3C).

*Microstegium vimineum* was capable of invading *P. pumila* in all soil by invasion history treatments, except for the sterile soil treatment from site WF. *Microstegium vimineum*’s ability to invade when rare increased in whole soil compared to sterile soil, supported by a significant main effect for the soil treatment (F=33.0053, p=0.0002, Table 4.1). *Pilea pumila* was capable of invading *M. vimineum* in two of the soil by invasion history treatments, sites HOF and CNSC with whole soil. *Pilea pumila*’s ability to invade when rare was not associated with the soil treatment, invasion history, or their interaction (Table 4.1).

None of the interaction coefficients were significantly associated with the soil treatment, invasion history, or their interaction at the p=0.05 level (Table 4.1). The interaction coefficient that represents the per capita effect of *M. vimineum* on *P. pumila* (\(a_{ji}\)) tended to increase in sterile soil compared to whole soil (Fig. 4.4), shown by a marginally significant main effect for the soil treatment (F=3.3381, p=0.0977, Table 4.1). The demographic and competition ratios were not associated with the soil treatment, invasion history, or their interaction (Table 4.1).

For both *M. vimineum* and *P. pumila*, per-germinant fecundity in the absence of competition (\(\lambda\)) was higher in sterile soil compared to whole soil and this difference increased across soil invasion history (Fig. 4.5A,B). This was evidenced through significant interactions.
between invasion history and the soil treatment (M. vimineum: F=7.1255, p=0.0235; P. pumila: F=6.5165, p=0.0287, Table 4.2). Soil treatment and invasion history were also independently associated with λ for M. vimineum (soil: F= 22.0056, p=0.0009; invasion history: F=6.0894, p=0.0332, Table 4.2) and P. pumila (soil: F=29.1795, p=0.0003; invasion history: F=9.3748, p=0.0120, Table 4.2).

Microstegium vimineum’s germination rate was higher in the sterile soil with shorter invasion histories, but as soil invasion history increased germination rate became higher in the whole soil treatment (Fig. 4.5C). This was supported by a marginally significant interaction between the soil treatment and invasion history (F=3.7443, p=0.0818, Table 4.2). Soil invasion history was also marginally significantly associated with M. vimineum’s germination rate (F=4.6665, p=0.0561, Table 4.2). Pilea pumila’s germination rate increased across soil invasion history (Fig 4.5D), shown by a marginally significant main effect for invasion history (F=3.3574, p=0.0968, Table 4.2).

Microstegium vimineum’s productivity (ƞ) was higher in sterile soil compared to whole soil and this difference increased across invasion history (Fig. 4.5E), supported by a significant interaction between the soil treatment and invasion history (F=5.6078, p=0.0394, Table 4.2). The soil treatment and invasion history were also significantly associated with M. vimineum’s productivity (soil: F=24.9004, p=0.0005; invasion history: F= 5.6078, p=0.0394, Table 4.2). Pilea pumila’s productivity increased in sterile soil compared to whole soil and increased across invasion history (Fig. 4.5F), shown by significant main effects for the soil treatment (F= 12.4001, p=0.0055, Table 4.2) and invasion history (F=5.0675, p=0.0481, Table 4.2).

Soil nutrient PCA axis 1 significantly increased across soil invasion history (F=5.9386, p=0.0350). Soil treatment had no overall effect on PCA axis 1, and did not interact with invasion
history. The 9 soil nutrient variables loaded negatively on PCA axis 1. The loadings of the 9 soil
variables onto PCA axis 1 varied from -0.0050 for phosphorous to -0.4811 for magnesium.
Magnesium, total % nitrogen, zinc, and potassium had the strongest loadings on PCA axis 1 of -
0.4811, -0.4614, -0.3979, and -0.3677, respectively. Soil nutrient PCA axes 2 and 3 were not
significantly associated with invasion history, soil treatment, or their interaction.

**Discussion**

Recent studies suggest that soil microbial communities, affect plant species relative
abundances and may promote coexistence within a community (Bever et al. 1997, Klironomos
2002, Bell et al. 2006, Kulmatiski et al. 2008, Mangan et al. 2010, MacDougall et al. 2011,
Bagchi et al. 2014). Coexistence within a community is disrupted by invasive species possibly
due to them having a less negative plant-soil feedback relative to native plant species in the
community (Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004b, Reinhart and
Callaway 2006). Here we found that soil biota increased the likelihood of coexistence between
invasive *M. vimineum* and native *P. pumila*, but this effect did not increase across soil invasion
history.

The likelihood of coexistence between invasive *M. vimineum* and native *P. pumila* increased
in the presence of soil biota, consistent with the predictions of current literature (Bever 1994,
Petermann et al. 2008). This effect occurred due to an increase in stabilizing niche differences
rather than shifts in average fitness differences, suggesting that soil biota had a stronger effect on
species' competitive interactions rather than species’ overall fitness. Breaking down niche
differences into the four different interaction coefficients (Fig. 4.4) we found that the increase in
stabilizing niche differences with whole soil was driven by a decrease in the effect of *M.*
*vimineum* on *P. pumila* and marginal increases in the effect of *M. vimineum* and *P. pumila* on themselves. Soil biota may have ameliorated the effect of *M. vimineum* on *P. pumila* either by suppressing the competitive effect of *M. vimineum* or promoting the competitive response of *P. pumila* (Goldberg 1996). Because we used soil conditioned by *M. vimineum*, the soil communities may have contained *M. vimineum* specific pathogens that suppressed its competitive effect on *P. pumila* (VanderPutten and Peters 1997, Hendriks et al. 2015). Alternatively, *P. pumila*’s competitive ability may have increased due to the presence of favorable mutualists. Both *P. pumila* and *M. vimineum* are known to interact with arbuscular mycorrhizal fungi (AMF) (Lankau 2013, Lee et al. 2014). How dependent and specific each species is in its AMF interaction is unknown, although many invasive species have facultative AMF strategies and therefore may rely less on these interactions compared to native species (Pringle et al. 2009). Soil biota increased the competitive effect of both species on themselves. *M. vimineum* or *P. pumila* specific pathogens and pests may have magnified intraspecific interactions if the pathogen populations responded exponentially to increasing host density.

Our finding that soil biota did not affect relative fitness differences between the two populations is contradictory to studies on plant-soil feedbacks that find reductions in individual plant performance due to conspecific cultured soil biota (Bever 1994, Packer and Clay 2000, Klironomos 2002, Mangan et al. 2010). Instead our study suggests soil biota increase the likelihood of coexistence by increasing the tendency of species to limit themselves more strongly than they limit other species. Assessing inter and intraspecific competition is not typical in plant-soil feedback experiments (Bever 2003), which typically focus on individual plant performance, but may be vital in evaluating microbial effects on plant coexistence (Callaway et al. 2004a, Casper and Castelli 2007, Shannon et al. 2012, Chiuffo et al. 2015).
Microstegium vimineum would competitively exclude P. pumila in 11 of the treatment combinations due to its higher average fitness and small stabilizing niche differences (Fig. 4.3A). In all 14 of the treatment combinations M. vimineum had higher average fitness relative to P. pumila, shown by average fitness ratios less than 1 in every combination (Fig. 4.3A). Additionally, in 9 of the treatment combinations the stabilizing niche differences were negative (destabilizing), due to the strong per capita effect of M. vimineum on P. pumila (α_{ji}), which was much stronger than M. vimineum’s effect on itself (α_{ii}). Microstegium vimineum’s strong competitive effect on P. pumila and higher average fitness exemplifies its invasiveness and is consistent with literature on M. vimineum’s superior competitive ability and its suppression of native plant growth (Leicht et al. 2005, Marshall et al. 2009, Flory and Clay 2010b, a, Bauer and Flory 2011).

While soil biota did not affect average fitness differences, specific vital rates for M. vimineum and P. pumila were affected by the soil treatment. Both M. vimineum and P. pumila’s per-germinant fecundity in the absence of competition (λ) and productivity (η), which includes germination rate (g) and λ in its calculation, were higher in sterile soil compared to whole soil. Because soil biota had a similar negative effect on both species’ vital rates, the changes did not have an overall effect on average fitness differences. The suppression of productivity and λ in whole soil indicates a generally antagonistic soil community, where the beneficial effects of mutualists are outweighed by the negative effects of pests and pathogens. Germination rate was not affected by the soil biota treatment.

Contrary to our predictions, the likelihood of coexistence did not increase across invasion history of the soil biota, although certain vital rates were associated with invasion history. Both M. vimineum and P. pumila’s λ increased across soil invasion history in sterile soil, while it
remained constant and lower, in whole soil across invasion history (Fig 4.5A,B). *Microstegium vimineum* and *P. pumila*’s germination rates increased across soil invasion history in both soil treatments (Fig. 4.5C,D). For *M. vimineum*, this pattern occurred only in the whole soil treatment. Patterns in η, which combine fecundity and germination rates, generally mirrored those in fecundity (λ) alone.

Multiple factors could explain the overall increase in *M. vimineum* and *P. pumila*’s η across invasion history in sterilized soil, including pre or post-invasion differences in the abiotic conditions of the soil across invasion history. For instance, *M. vimineum* invasion has been associated with changes in soil nutrients and pH (Kourtev et al. 2003, McGrath and Binkley 2009, Craig et al. 2015, Tekiela and Barney 2015). Our analysis of soil nutrients indicated consistent changes across soil invasion history, but these changes generally indicated a reduction in nutrient concentrations. Thus, it remains unclear why sterilized versions of long-invaded soils promoted the performance of both species. *Microstegium vimineum* and *P. pumila*’s η remained constantly lower in whole soil suggesting that soil biota may suppress the effects of fluxes in abiotic conditions on plant performance. Our results suggest that the soil biota’s ability to suppress η is increasing across invasion history, since the vital rate remained constant in whole soil while it increased in sterile soil. This could be due to an accumulation of antagonistic soil communities across invasion history driven by *M. vimineum* that have a negative effect on native plant species as well (Beckstead et al. 2010, Mordecai 2013, Li et al. 2014).

Both plant species in our experiment have similar life history strategies and possibly share similar soil microbial communities, which may explain the similarity in their responses (Johnson et al. 2012, Suding et al. 2013). Native species with varying life history strategies or habitat preferences may respond differently to *M. vimineum* condition soil communities, with
potentially different coexistence outcomes. In this experiment, we were specifically interested in the potential for coexistence driven by soil biota in already invaded soil, but future work should consider using conspecific conditioned soil from all interacting species to obtain a full understanding of the facilitation of coexistence by soil biota.

Overall our results demonstrate that soil communities are essential to promoting coexistence between our invasive and native plant species, and to our knowledge this is the first rigorous assessment of the soil community’s role in plant species coexistence. Phenomenological studies have found coexistence of plant species to be strongly stabilized in multiple systems, but the mechanisms behind this pattern remain unclear (Levine and HilleRisLambers 2009, Adler et al. 2010, Chu and Adler 2015). While decades of research have evaluated the importance of resource competition and niche partitioning for plant species interactions (Grime 1979, Tilman 1982), interactions between plants and diverse soil microbial communities may represent an equally powerful force maintaining the high diversity seen in many plant communities. Combining experimental manipulations with population dynamic modeling within the modern coexistence framework provides a powerful approach to gaining insight into the mechanisms maintaining plant diversity in nature, and similar studies in other environments are necessary to determine whether soil microbes play a consistent role across plant communities.

References


Table 4.1 - Statistical results of linear models testing the effect of soil invasion history (years), soil biota treatment (whole versus sterile), and their interaction on various response variables that are related to coexistence between *Microstegium vimineum* and *Pilea pumila*. $R^2$ is the overall model fit.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model term</th>
<th>F value</th>
<th>p value</th>
<th>$R^2$</th>
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<td>effect of <em>M. vimineum</em> on <em>P. pumila</em> $(\alpha_{ji})$</td>
<td>invasion history</td>
<td>1.1406</td>
<td>0.3106</td>
<td>0.3944</td>
</tr>
<tr>
<td></td>
<td>soil biota</td>
<td>3.3381</td>
<td>0.0977 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>2.0341</td>
<td>0.1843</td>
<td></td>
</tr>
<tr>
<td><em>M. vimineum</em> 's ability to invade $(\alpha'<em>{ij} - \alpha'</em>{ji})$</td>
<td>invasion history</td>
<td>0.1525</td>
<td>0.7043</td>
<td>0.7701</td>
</tr>
<tr>
<td></td>
<td>soil biota</td>
<td>33.0053</td>
<td>0.0002 ***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>0.3464</td>
<td>0.5692</td>
<td></td>
</tr>
<tr>
<td><em>P. pumila</em> 's ability to invade $(\alpha'<em>{ii} - \alpha'</em>{jj})$</td>
<td>invasion history</td>
<td>0.0021</td>
<td>0.9644</td>
<td>0.03073</td>
</tr>
<tr>
<td></td>
<td>soil biota</td>
<td>0.2666</td>
<td>0.6168</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>0.0484</td>
<td>0.8304</td>
<td></td>
</tr>
<tr>
<td>demographic ratio</td>
<td>invasion history</td>
<td>0.8958</td>
<td>0.3662</td>
<td>0.2652</td>
</tr>
<tr>
<td></td>
<td>soil biota</td>
<td>2.6667</td>
<td>0.1335</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>0.0467</td>
<td>0.8333</td>
<td></td>
</tr>
<tr>
<td>competition ratio</td>
<td>invasion history</td>
<td>0.7356</td>
<td>0.4112</td>
<td>0.2241</td>
</tr>
<tr>
<td></td>
<td>soil biota</td>
<td>1.2579</td>
<td>0.2883</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>0.8944</td>
<td>0.3666</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2- Statistical results of linear models testing the effect of soil invasion history (years), soil biota treatment (whole versus sterile), and their interaction on specific vital rates for *Microstegium vimineum* and *Pilea pumila*. $R^2$ is the overall model fit.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model term</th>
<th>F value</th>
<th>p value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. vimineum</em></td>
<td>invasion history</td>
<td>6.0894</td>
<td>0.0332</td>
<td>**</td>
</tr>
<tr>
<td>fecundity ($\lambda_i$)</td>
<td>soil biota</td>
<td>22.0056</td>
<td>0.0009</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>7.1255</td>
<td>0.0235</td>
<td>**</td>
</tr>
<tr>
<td><em>P. pumila</em></td>
<td>invasion history</td>
<td>9.3748</td>
<td>0.0120</td>
<td>**</td>
</tr>
<tr>
<td>fecundity ($\lambda_i$)</td>
<td>soil biota</td>
<td>29.1795</td>
<td>0.0003</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>6.5165</td>
<td>0.0287</td>
<td>**</td>
</tr>
<tr>
<td><em>M. vimineum</em></td>
<td>invasion history</td>
<td>4.6665</td>
<td>0.0561</td>
<td>*</td>
</tr>
<tr>
<td>germination rate</td>
<td>soil biota</td>
<td>1.1703</td>
<td>0.3047</td>
<td></td>
</tr>
<tr>
<td>($g_i$)</td>
<td>history*biota</td>
<td>3.7443</td>
<td>0.0818</td>
<td>*</td>
</tr>
<tr>
<td><em>P. pumila</em></td>
<td>invasion history</td>
<td>3.3574</td>
<td>0.0968</td>
<td>*</td>
</tr>
<tr>
<td>germination rate</td>
<td>soil biota</td>
<td>1.7683</td>
<td>0.2131</td>
<td></td>
</tr>
<tr>
<td>($g_i$)</td>
<td>history*biota</td>
<td>1.2334</td>
<td>0.2927</td>
<td></td>
</tr>
<tr>
<td><em>M. vimineum</em></td>
<td>invasion history</td>
<td>8.1697</td>
<td>0.0170</td>
<td>**</td>
</tr>
<tr>
<td>productivity ($\eta_i$)</td>
<td>soil biota</td>
<td>24.9004</td>
<td>0.0005</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>5.6078</td>
<td>0.0394</td>
<td>*</td>
</tr>
<tr>
<td><em>P. pumila</em></td>
<td>invasion history</td>
<td>5.0672</td>
<td>0.0481</td>
<td>*</td>
</tr>
<tr>
<td>productivity ($\eta_i$)</td>
<td>soil biota</td>
<td>12.4001</td>
<td>0.0055</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>history*biota</td>
<td>2.1441</td>
<td>0.1738</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.1 - Map of *Microstegium vimineum* invasion history, created using the kriging function in ArcMap from ArcGIS 10.1. Points on map are the 7 soil collection sites and are also listed in the table with their abbreviated identification, state of location, and estimated time since invasion.

<table>
<thead>
<tr>
<th>Site</th>
<th>State</th>
<th>Time since invasion (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>IN</td>
<td>11</td>
</tr>
<tr>
<td>HOF</td>
<td>NC</td>
<td>16</td>
</tr>
<tr>
<td>CHOC</td>
<td>AL</td>
<td>20</td>
</tr>
<tr>
<td>SC</td>
<td>MD</td>
<td>26</td>
</tr>
<tr>
<td>CNSC</td>
<td>VA</td>
<td>35</td>
</tr>
<tr>
<td>WF</td>
<td>GA</td>
<td>43</td>
</tr>
<tr>
<td>DF</td>
<td>NC</td>
<td>47</td>
</tr>
</tbody>
</table>
Figure 4.2 - Layout of the response surface design for the greenhouse experiment. The first column and top row show the range of densities of Microstegium vimineum (MV) and Pilea pumila (PP) respectively, that were used in the experiment. All density combinations within the response surface were represented once in each of the 14 invasion history by soil treatment combinations, except for the combination where one M. vimineum or P. pumila individual grew alone, which was replicated 5 times. The density combinations below have the density of M. vimineum as the first number in the pair and then the density of P. pumila as the second number.

<table>
<thead>
<tr>
<th>MV=0</th>
<th>PP=0</th>
<th>PP=1</th>
<th>PP=4</th>
<th>PP=7</th>
<th>PP=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV=0</td>
<td>0, 1</td>
<td>0, 4</td>
<td>0, 7</td>
<td>0, 10</td>
<td></td>
</tr>
<tr>
<td>MV=1</td>
<td>1, 0</td>
<td>1, 1</td>
<td>1, 4</td>
<td>1, 7</td>
<td>1, 10</td>
</tr>
<tr>
<td>MV=4</td>
<td>4, 0</td>
<td>4, 1</td>
<td>4, 4</td>
<td>4, 7</td>
<td>4, 10</td>
</tr>
<tr>
<td>MV=7</td>
<td>7, 0</td>
<td>7, 1</td>
<td>7, 4</td>
<td>7, 7</td>
<td>7, 10</td>
</tr>
<tr>
<td>MV=10</td>
<td>10, 0</td>
<td>10, 1</td>
<td>10, 4</td>
<td>10, 7</td>
<td>10, 10</td>
</tr>
</tbody>
</table>

Figure 4.2- Layout of the response surface design for the greenhouse experiment. The first column and top row show the range of densities of Microstegium vimineum (MV) and Pilea pumila (PP) respectively, that were used in the experiment. All density combinations within the response surface were represented once in each of the 14 invasion history by soil treatment combinations, except for the combination where one M. vimineum or P. pumila individual grew alone, which was replicated 5 times. The density combinations below have the density of M. vimineum as the first number in the pair and then the density of P. pumila as the second number.
Fig 4.3- Blue points are the whole soil treatment, green points are the sterile soil treatment. Trend lines and R² values correspond to the soil type with the same color. Panel A: The 14 invasion history x soil type treatment combinations plotted based on their stabilizing niche and average fitness differences. Black lines represent the barrier between coexistence and competitive exclusion. Coexistence between *Microstegium vimineum* and *Pilea pumila* is possible in the area with vertical hash marks, priority effects occur in the area with diagonal hash marks. Competitive exclusion by *M. vimineum* would occur in the space below the black lines and competitive exclusion by *P. pumila* would occur in the space above the black lines. Panel B: The 14 treatment combinations plotted based on their distance to coexistence and *M. vimineum* invasion history. Panel C: The 14 treatment combinations plotted based on their stabilizing niche differences and *M. vimineum* invasion history. D: The 14 treatment combinations plotted based on their average fitness ratio and *M. vimineum* invasion history.
Figure 4.4 - The average strength of the 4 interaction coefficients across soil type, blue bars are the whole soil treatment, green bars are the sterile soil treatment. $\alpha_{ji}$ is the per capita effect of *Microstegium vimineum* on *Pilea pumila*. 
Figure 4.5- Blue points are the whole soil treatment, green points are the sterile soil treatment. Trend lines and $R^2$ values correspond to the soil type with the same color. Panel A and B: The 14 invasion history x soil type treatment combinations plotted based on *Microstegium vimineum/Pilea pumila*’s fecundity in the absence of competition and *M. vimineum* invasion history. Panel C and D: The 14 treatment combinations plotted based on *M. vimineum/P. pumila*’s germination rate and *M. vimineum* invasion history. Panel E and F: The 14 treatment combinations plotted based on *M. vimineum/P pumila*’s productivity and *M. vimineum* invasion history.
CHAPTER V: CONCLUSION

Coexistence and plant-soil microbe interactions

In classical plant ecology theory, coexistence and the relative abundance of plant species within communities have been explained by resource competition. Species dominance or rarity is driven by their ability to acquire resources relative to other species and coexistence is stabilized when species differ in their resource requirements or the space and timing of their resource acquisition (Grime 1979, Tilman 1982). More recently, plant-soil feedbacks, driven by the soil microbial community, have been proposed as a mechanism for coexistence, due to their ability to drive negative frequency dependence within plant species (Bell et al. 2006, Bever et al. 2010, Bagchi et al. 2014) and their correlation to plant species’ relative abundances (Bever et al. 1997, Klironomos 2002, Kulmatiski et al. 2008, Mangan et al. 2010, MacDougall et al. 2011). Our results, from Chapter IV, confirm that the soil community promotes coexistence, specifically through increased stabilizing niche differences, and to our knowledge this is the first thorough assessment of the soil biota’s role in species coexistence using the contemporary coexistence framework (Chesson 2000).

Our finding that soil biota did not affect relative fitness differences between the two populations is contradictory to studies on plant-soil feedbacks that find reductions in individual plant performance due to conspecific cultured soil biota (Bever 1994, Packer and Clay 2000, Klironomos 2002, Mangan et al. 2010). Instead our study suggests soil biota increase the likelihood of coexistence by increasing the tendency of species to limit themselves more strongly
than they limit other species. Assessing inter and intraspecific competition is not typical in plant-soil feedback experiments (Bever 2003), which typically focus on individual plant performance, but may be vital in evaluating microbial effects on plant coexistence (Callaway et al. 2004a, Casper and Castelli 2007, Shannon et al. 2012, Chiuffo et al. 2015).

In our study we were specifically interested in the potential for coexistence driven by soil biota in already invaded soil, but future work should consider using conspecific conditioned soil from all interacting species to obtain a full understanding of the facilitation of coexistence by soil biota. Our results show that the overall soil community increases the likelihood of coexistence, but who the exact drivers are within the community is unknown. Future work is needed to understand the particular drivers within the diverse soil community.

**Coexistence across invasion history**

When invasive species enter communities they can disrupt species coexistence and are associated with declines in resident species diversity and abundance (Vila et al. 2011). Invaders’ superior competitive ability (Vila and Weiner 2004) and plant community dominance could be due to their lack of coevolutionary history in the invaded range (Hallett 2006). The advantage, through evolutionary novelty, could come in the form of novel weapons (Hierro and Callaway 2003, Callaway and Ridenour 2004, Zheng et al. 2015), unique niche requirements (Lloret et al. 2005, Godoy et al. 2009, Brym et al. 2011), or enemy release (Mitchell and Power 2003, DeWalt et al. 2004, Liu and Stiling 2006). Whether this advantage persists through invasion time is unclear; ecological or evolutionary forces could develop to reduce competitive exclusion and promote coexistence (Strayer et al. 2006, Lankau et al. 2009, Iacarella et al. 2015).

Our results, particularly from Chapters II and III, suggest that coexistence between invasive *M. vimineum* and native plants is more likely at sites with a longer invasion history compared to
sites with a short invasion history. In the observational study, *M. vimineum*’s survival at low frequency declined across invasion history, suggesting that per capita population growth rate when rare is declining through invasion time. A decline in per capita population growth rate at low frequency will make it more difficult for *M. vimineum* to persist when rare within a community (Chesson 2000, Adler et al. 2007), making its population more vulnerable to extinction due to size fluctuations. In the field experiment, *M. vimineum*’s negative frequency dependence increased across invasion history, suggesting that stabilizing forces are accumulating. An increase in negative frequency dependence would stabilize *M. vimineum*’s population and potentially reduce competitive exclusion of native plants. These results combined suggest that at sites with short invasion history coexistence between *M. vimineum* and native plants is less likely due to reduced population stabilizing forces for *M. vimineum* and invasion persistence is more likely due to higher population growth rates when rare. Conversely, at sites with longer invasion history the likelihood of invasion persistence is reduced and *M. vimineum*’s population has accumulated stabilizing forces, promoting coexistence between it and native plants.

In our field experiment we competed *M. vimineum* with only one native plant, *P. pumila*. Repeating the field experiment with other combinations of native plant species would better confirm patterns across invasion history for *M. vimineum*. Although, we would expect patterns in frequency dependence to be consistent since this phenomenon appears to be *M. vimineum* specific.

**Mechanism behind changes in coexistence across invasion history**

Since the soil microbial community may promote coexistence through negative plant-soil feedbacks, invader dominance may arise due to a tendency for invaders to have neutral or
positive plant-soil feedbacks (Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004b). A more positive plant-soil interaction in the invaded range could be due to an absence of specialized enemies or novel mutualistic interactions (Reinhart and Callaway 2006). A more positive plant-microbe interaction could promote competitive exclusion by the invader through weak negative frequency dependence and high average fitness relative to native plants in the community. A soil microbe community with a long invasion history has potentially accumulated specialized pests and pathogens (Hawkes 2007, Mitchell et al. 2010, Schultheis et al. 2015) causing for a more negative plant-soil feedback (Diez et al. 2010, Dostal et al. 2013). A more negative plant-soil microbe interaction could promote coexistence between the invader and the native community through increased stabilizing forces, i.e. negative frequency dependence, and reduced average fitness.

In Chapter II and III we found evidence that suggests the changes in *M. vimineum*’s population dynamics across invasion history may arise from the accumulation of a more antagonistic soil community. In our observational study we found changes in the general fungal community on *M. vimineum*’s roots and in invaded soil across invasion history. In the field experiment the pattern of increasing negative frequency dependence across invasion history for *M. vimineum* was primarily in conspecific conditioned soil. *Microstegium vimineum* soil conditioning could be promoting the accumulation of *M. vimineum* specific pests and pathogens across invasion history (Diez et al. 2010, Dostal et al. 2013). This accumulation of soil pests could increase stabilizing forces for *M. vimineum* and reduce its invasiveness at sites with longer invasion history (Strayer et al. 2006, Flory and Clay 2013). However, in greenhouse conditions we found no evidence that soil communities from older invaded sites exerted stronger effects.
than those from younger invaded sites. Future work is necessary to experimentally define a direct link between changes in soil community composition and population dynamics of *M. vimineum*.

**References**


