

**Hidden Stories of the Ground Layer: Potential Mechanisms Driving Community Changes
in Invertebrates due to *Microstegium vimineum***

Courtney Hill

Under the supervision of Dr. Justin Wright,
Department of Biology, Duke University

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Research Supervisor

Faculty Reader

Director of Undergraduate Studies

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Abstract

Invasive plants, when successful, outcompete natives and can result in major reductions in local botanical biodiversity. In consequence, the altered plant species composition following invasion can induce change in higher trophic levels. In the southeastern United States, a widespread case of an invasive plant is *Microstegium vimineum* or Japanese Stiltgrass. Since invertebrate community effects of *M. vimineum* have varied across studies, this research investigates potential mechanisms leading to *M. vimineum* effects on higher trophic levels. Invertebrates were caught using pitfall traps in 4 types of treatment plot: control plots (naturally uninvaded by the grass), MV plots (fully covered by the grass), MVR plots (previously covered by the grass but *M. vimineum* then removed), and shade plots (uninvaded by the grass and covered by two layers of black mesh material). Taxa, feeding groups, and size categories (length in mm) were each analyzed for significantly different responses (measured by quantity) to the different treatment types. Five of these groups showed significant, with $p < 0.5$, differences in quantity between treatment types: Cosmetidae, Formicidae, Spirobolidae, omnivores, and predators. Since the groups varied in how the treatments affected them, the results of this study emphasize the importance of understanding that the same stimuli can be beneficial, harmful, or neutral to different groups. Therefore, the effects of invasive plants on the overall invertebrate community are dependent upon the taxa that comprise the invertebrate community.

Introduction

Overview

Aggressive and alien (in combination, *invasive*) plants have long been of interest to ecologists, because when successful, they outcompete native plants and can result in major reductions in local biodiversity (Hejda et al. 2009, Paudel et al. 2017, Gaertner et al. 2009). In consequence, the altered plant species composition following invasion can induce change in higher trophic levels through mechanisms that are unlikely to be universal across species and communities. In the southeastern U.S., *Microstegium vimineum* (Japanese Stiltgrass) is a widespread invasive species that provides the opportunity to examine mechanisms leading to changes in higher trophic levels. My research investigates potential mechanisms leading to *M. vimineum*'s effects on invertebrates as a way to help explain why these overall effects have varied among previous studies. In doing so, this study aims to clarify potential ways invasive plants may be interacting with higher trophic levels.

Invasive species and effects in other trophic levels

Here, I highlight two points that provide an argument for a mechanistic and taxon-specific approach to understanding invasive plant relationships with invertebrates. Firstly, changes in invertebrate populations can occur through much more than one possible mechanism resulting from plants, including soil-induced changes, architectural mechanisms, possible trophic mechanisms, among other possibilities. Secondly, these varied mechanisms don't simply affect all taxa across all trophic levels in the same way.

Hejda et al. (2017) found that areas invaded by *Robinia pseudoacacia* showed significantly decreased nocturnal Lepidopteran (butterfly) species richness and significant differences in species composition in Lepidopterans, birds, and plants relative to uninvaded sites,

demonstrating different responses to invasives among several taxa from different trophic levels. Another study found that *Alliaria petiolate* (garlic mustard) fruit structures were found to relate significantly with increased web-building spider populations, and removal of the fruit structures caused decreased abundances of these spiders (Smith-Ramesh 2017). In this case, we see a structural mechanism acting on these spider taxa. Finally, Zhang et al. (2019) found that invasive plants had increased mycorrhiza (symbiont fungus) but reduced abundances of herbivores and predators relative to native plants, as a result of rhizosphere effects (below-ground changes caused by plant roots). Thus, in this last example, we see an additional mechanism by which plants affect other trophic levels, and we see differing reactions to the same mechanism.

Given this evidence that multiple possible mechanisms caused by plant community changes can produce higher trophic-level community changes, a taxon-specific approach allows detection of different mechanisms affecting different taxa, since the above research showed that various taxa don't always react in the same way to the same stimuli. Also, it provides an argument for studying invertebrates, since higher trophic levels can be directly affected by changes in the primary producers.

Microstegium vimineum: A case study for invasive plant/invertebrate relationships

M. vimineum is an invasive grass that was introduced to the United States around 1920. In this short time, it has spread to 24 eastern and southeastern states. In addition to the U.S., it is considered an invasive in Europe, Africa, Australia, island countries, and other locations. *M. vimineum* fixes carbon via the C4 pathway, which makes it more tolerant to high temperatures, sunlight, limited nitrogen/CO₂, and drought. Uncharacteristically for a C4 plant, *M. vimineum* is also adapted to shade. These traits, combined with quick growth, abundant fruiting, and lack of browsing by animals, notably deer (Duguay and Farfaras 2011), allow *M. vimineum* to negatively

impact growth of native plants (Marshall and Buckley 2009), making it an interesting case study for invasive plants as a whole, but also an important system to understand on its own.

Though *M. vimineum* relationships with other primary producers is a well-studied area of ecological research, much remains to be investigated within the relationships between it and higher trophic levels, such as invertebrates. Not only are ground-level invertebrates a simpler study case in that abundant observations can be made via collection due to small size, high numbers, and ground-dwelling nature (versus flying), but also the understory where *M. vimineum* persists likely plays a more direct role in invertebrates in the form of habitat and nutrients than in animals that occupy other levels. A final reason for the study of invertebrates is that they are important bioindicators, with various taxa indicating qualities about particular levels of understory habitat. For instance, spider abundances have been used in studies to determine environmental toxin levels, harvestman absence can help indicate disturbance, and grasshopper changes are thought to be indicators of change in grasslands (Gerlach et al. 2013).

Research purpose and approach

Past literature is inconclusive in regard to whether *M. vimineum* has a generally negative, positive, or variable effect on invertebrates. Marshall and Buckley et al. (2009), showed that transects in *M. vimineum*-invaded areas had significantly more true crickets (Gryllidae) and leafhoppers (Cicadellidae) and significantly fewer roaches (Blatellidae) and leaf beetles (Chrysomelidae) relative to uninvaded areas. However, their study concluded that unlike many invasives which directly impact insect diversity and abundance, *M. vimineum* presence plays less of a role in insect diversity and abundance than overall plant community diversity does. An earlier paper by the same authors in 2007, also an observational paper using transects, found an overall increase in herbivores, carnivores, scavengers, and omnivores that the researchers

attributed to the 2.5x greater cover by *M. vimineum* (Marshall 2007). In contrast, Simao et al. (2010) found an overall *reduction* in invertebrate abundance and richness after introducing the grass, as well as a significant reduction specifically in carnivore abundance and richness.

Proposed mechanisms for changes in invertebrate communities include differences under *M. vimineum* in litter decomposition, soil changes, shade, and temperature (Marshall and Buckley 2009, McGrath and Binckley 2009, Civitello et al. 2008), with litter decomposition likely occurring as a result of the other mechanisms (Chen et al. 2014). With this in mind, this study used 4 types of treatment plots: control plots (naturally uninvaded by the grass), MV plots (fully covered by the grass naturally), MVR plots (previously covered by *M. vimineum*, but then *M. vimineum* was removed), and shade plots (uninvaded by the grass and then covered above by two layers of black mesh material to mimic shade provided by full cover of *M. vimineum*). Invertebrates were captured in each plot type, and resulting abundances were used to help draw conclusions about possible acting mechanisms. Having mechanistic information may help us understand why different experiments show different trends and can help narrow down what manipulations result in particular changes in taxon-specific or overall abundance and diversity.

Considering the background, my initial expectation was that a structural property of *M. vimineum*, such as its carpeting that may lead to temperature changes, was the primary driver of changes in invertebrate communities. Past experiments may have varied in *M. vimineum* density, which could have led to different results due to a mechanism like structural or temperature differences. On the other hand, if there had been a soil-level change, invertebrates would have reacted similarly throughout experiments because I supposed that soil differences under varied *M. vimineum* densities would likely be a less drastic than structure or temperature differences caused by density variation. Additionally, I expected that different taxa would react in different

ways, just as different taxa of invertebrates have had varying reactions to other mechanisms such as shade, moisture, and elevation (Mokany et al. 2008, Brygadyrenko 2014, Davis et al. 2006).

Methods

Four sites that included areas invaded by *M. vimineum* and uninvaded areas were chosen from the Duke Forest in Durham, NC. At each site, four types of 1 meter by 1-meter research plot were established. Plots were established three months before traps were set, over which time, plots were not meticulously maintained. During sampling, treatments were upkept several times a week.

- 1) C- (Control) plots in the site naturally uninvaded by the grass
- 2) MV- (*M. vimineum*) plots fully covered by the grass naturally
- 3) MVR- (*M. vimineum* with removal) plots that were previously covered by the grass but were experimentally hand-weeded leaving only the sparse natives that were in the plot. (Whether a plot was to be an MV plot or and MVR plot was assigned at random.)
- 4) S- (Shade) plots uninvaded by the grass and then covered above by two layers of black mesh material to mimic shade provided by full cover of *M. vimineum*. (Whether a plot was to be a C or an S plot was assigned at random.)

The MVR type of plot was intended to observe whether soil changes are a possible mechanism of effect upon invertebrate communities. Similar differences from the control plots in both MV and MVR plots, but not differences between the S plots and the control plots would theoretically suggest soil as a cause of the differences, rather than shade.

The S plot was intended to observe whether shade differences are a possible mechanism of effect. Similar differences from the control plots in MV and S plots, but not in MVR plots would theoretically suggest shade or shade related effects as a cause of the differences.

At each site, one block consisted of all four plot types, though they were not treated as a block in this study's statistical analysis (see discussion). There were 3 such replicates per site with the plots in each replicate placed closely, to minimize any outside differences such as elevation change. Thus, each of 4 sites contained 12 plots, for a total of 48 plots.

Pitfall traps were the method of collection: In each corner of the plots, cups were placed into the ground with their brims even with ground level. The cups were filled to 50 mL with isopropyl alcohol. For three weeks, from late September to early October in 2017, traps were set on Tuesday afternoon and then collected on Thursday afternoon, giving each set of cups approximately 48 hours to accumulate. The four cups from each corner of a plot were combined into one sample cup during collection.

After collection, the specimens were filtered through stacked 0.5 mm and 2 mm sieves. Material caught by each sieve was cleaned in and stored in ethanol then placed for storage into a cold room. For this study, we did not investigate the microinvertebrates caught by the finer sieve and focused on invertebrates that were sized 2 mm (consistent with most of the Formicidae specimens).

These 2+ mm specimens were then categorized taxonomically, logged into their respective size categories (2-4 mm, 4-8 mm, 8-16 mm, 16-32 mm, or 32+ mm in length), and separated into feeding class (detritivore, herbivore, omnivore, predator) in accordance with the feeding class of their taxon. In most cases, specimens were categorized to family. Some groups were categorized to broader categories in cases when more specific identification could not be made or their taxa

were not the focus of this study, such as invertebrates that were not ground and low-vegetation dwelling (Lepidopterans, which are flying invertebrates; Isopoda which are a diverse and difficult group to classify, etc.). The result of this was analysis of 42 groups, outlined in Table 1.

Table 1. Groups analyzed for significant quantities of specimens in C, MV, MVR, or S

Taxa (31 Groups)

Class Arachnida

- Family Lycosidae
- Family Agelenidae
- Family Gnaphosidae
- Family Cosmetidae
- Family Sclerosomatidae

Class Diplopoda

- Order Spirobolida
- Family Polydesmidae
- Family Parajulidae
- Genus Oxidus

Class Gastropoda (w/shell)

Order Blattodea (non-termite)

Order Diptera

Order Hymenoptera (non-ant)

Order Isopoda

Feeding Groups (4 Groups)

- Detritivores
- Herbivores
- Omnivores
- Predators

Body length in mm (5 groups)

- 2-4
- 4-8
- 8-16
- 16-32
- 32+

Other measures (2 Groups)

- Specimen Count
- Taxon Richness

Order Orthoptera

- Family Gryllidae
- Subfamily Nemobiinae
- Subfamily Ceuthophilinae
- Genus Gryllus
- Genus Velarifictorus

Order Microcoryphia

- Family Meinertellidae

Superfamily Scarabaeoidea

- Family Geotrupidae
- Genus Onthophagus

Family Curculionoidea

Family Formicidae

Subfamily Harpalinae

Subfamily Tachyporinae

Though other taxa were identified from the specimens collected, taxa with fewer than 10 individuals and fewer than 3 trapping occurrences were disregarded to prevent false significant results resulting from a small sample size, though often these individuals were included by being a part of a broader category. For instance, a taxon identified was the Gryllidae subfamily Hapithinae, and though this taxon was not evaluated for significance individually, its numbers were represented in the Gryllidae group.

Statistical analysis was done in R. For each group in Table 1, including each taxon, size class, feeding group, total species richness and total abundance, I created Poisson-distributed (common for count data), full and reduced models using the **glmer** function in the **lme4** package in R. The full model included the fixed effect of treatment as well as the random effects of site and trial, and was compared to a reduced model that was the same except for the exclusion of treatment as a fixed effect. Therefore significant difference between the two models would indicate a significant difference in the models caused by treatment.

Before running these comparisons, I checked the models for overdispersion, which is when the data demonstrate more variability than would be expected for a given model (in this case, the Poisson distribution). I used the function **dispersion_glmer** in R package **blmeco** to test for overdispersion and corrected over-dispersed models by adding an observation-level random effect to the model as described in Harrison (2014).

After correcting for overdispersion, comparisons of the full and reduced Poisson-distributed mixed-effects models for each group were carried out using the function **anova** in the **stats** package in R. I compared the models for each group identified in Table 1, examining whether the experimental treatments affected which taxa were collected, size classes, feeding groups, total species richness, or total abundance. After significant ($p < 0.05$) results were found

in the anovas, I ran post-hoc Tukey tests (**TukeyHSD** function in **stats** package) on the full models in which treatment was significant. The results of these tests made it possible to determine which exact treatments were responsible for the overall significance observed in each group.

As another check for more complex relationships in overall composition, we conducted a non-metric multidimensional scaling (NMDS) ordination on the full data set using the function **metaMDS** in the R package, **vegan**. An NMDS is a way to visually compare compositions. In this study, each sample cup was visualized as a point on a graph, in which close points have a more similar composition. In basic terms, the program runs possible arrangements of the samples in space and continues to run possible arrangements until the points are plotted with minimum distance between similar points, although a max number of arrangement attempts can be specified. When the “stress” data is below 0.05 for an NMDS, this is considered an ideal optimization.

Results

Group significance

Of the forty-two groups analyzed, five of them showed significant differences between the four experimental treatments that were confirmed in Tukey post hoc tests. Five other groups' ANOVA's detected relationships, but post hoc testing of the individual relationships showed that while these groups did have relationships with p values below .1, they were only marginally significant. These marginally significant groups were not investigated for variance patterns. Interestingly, only three significant patterns of treatment differences were observed among the five significant groups. They are outlined below.

1. *MV plots have significantly more individuals than C and S plots.*

The families Spirobolidae (a millipede family) and Cosmetidae (armored harvestmen, an arachnid) both demonstrated higher abundances in invaded plots (Figure 1).

As seen in Table 2 and 3, between these two groups, Cosmetidae showed stronger significance than Spirobolidae, as indicated by lower p-values for both significant relationships (MV>C and MV>S) in Cosmetidae. Though not significant ($p < 0.05$), it is also worth noting that in Cosmetidae MVR was nearly significantly greater than C and S plots as well.

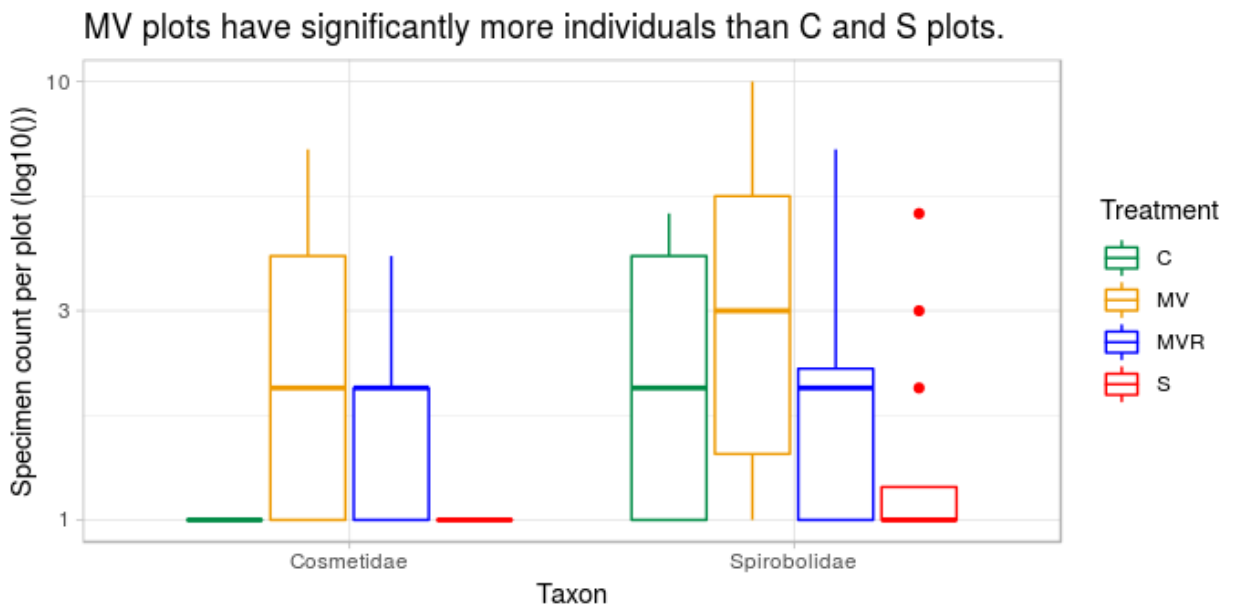


Figure 1. Spirobolidae (a millipede family) and Cosmetidae (a harvestman family) demonstrate higher abundances in *M. vimineum*-covered plots than in non-invaded plots and plots in which shade treatments were applied.

Table 2. Cosmetidae results

| Linear Hypotheses: | | | | |
|--------------------|----------|------------|---------|------------|
| | Estimate | Std. Error | z value | Pr(> z) |
| MV - C == 0 | 3.2189 | 1.0197 | 3.157 | 0.00798 ** |
| MVR - C == 0 | 2.5649 | 1.0377 | 2.472 | 0.05376 . |
| S - C == 0 | 0.6931 | 1.2246 | 0.566 | 0.57139 |
| MVR - MV == 0 | -0.6539 | 0.3419 | -1.913 | 0.11160 |
| S - MV == 0 | -2.5257 | 0.7348 | -3.437 | 0.00352 ** |
| S - MVR == 0 | -1.8718 | 0.7595 | -2.465 | 0.05376 . |

Table 3. Spirobolidae results

| Linear Hypotheses: | | | | |
|--------------------|----------|------------|---------|----------|
| | Estimate | Std. Error | z value | Pr(> z) |
| MV - C == 0 | 0.6931 | 0.2642 | 2.624 | 0.0435 * |
| MVR - C == 0 | 0.2877 | 0.2853 | 1.008 | 0.6267 |
| S - C == 0 | -0.1001 | 0.3130 | -0.320 | 0.7491 |
| MVR - MV == 0 | -0.4055 | 0.2411 | -1.681 | 0.3707 |
| S - MV == 0 | -0.7932 | 0.2733 | -2.903 | 0.0222 * |
| S - MVR == 0 | -0.3878 | 0.2938 | -1.320 | 0.5606 |

2. *S* plots have significantly fewer individuals than C, MV, and MVR plots.

The family Formicidae (ants) and all combined omnivores both demonstrated significantly lower values for *S* plots than all of the other types (Figure 2). Between these two taxa, the omnivore results showed stronger significance, as indicated by lower *p*-values for all significant relationships ($S < C$, $S < MV$, and $S < MVR$) in the omnivores (Table 4 and 5). This stronger relationship in omnivores may especially be notable in eliminating Formicidae (which was categorized as an omnivorous taxon) as the sole cause of the same pattern in omnivores, which was my first instinct since the Formicidae individuals were so numerous. However, since the omnivore significance is even stronger, it seems reasonable to suggest that other omnivorous groups in addition to Formicidae contributed to the significance observed in omnivores overall.

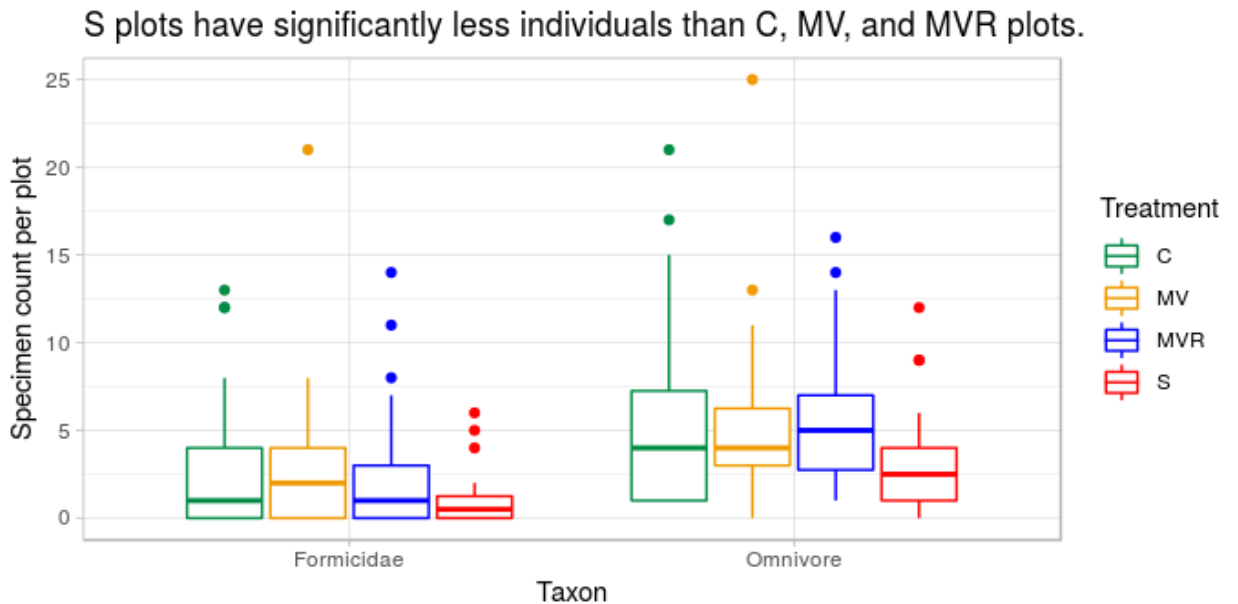


Figure 2. Formicidae (ants) and omnivores demonstrate lower abundances in plots in which shade treatments were applied than in all other treatment types.

Table 4. Formicidae results

| Linear Hypotheses: | | | | |
|--------------------|----------|------------|---------|------------|
| | Estimate | Std. Error | z value | Pr(> z) |
| MV - C == 0 | 0.07858 | 0.24532 | 0.320 | 1.00000 |
| MVR - C == 0 | 0.00335 | 0.24683 | 0.014 | 1.00000 |
| S - C == 0 | -0.80595 | 0.27631 | -2.917 | 0.01657 * |
| MVR - MV == 0 | -0.07523 | 0.24396 | -0.308 | 1.00000 |
| S - MV == 0 | -0.88453 | 0.27383 | -3.230 | 0.00742 ** |
| S - MVR == 0 | -0.80930 | 0.27556 | -2.937 | 0.01657 * |

Table 5. Omnivore results

| Linear Hypotheses: | | | | |
|--------------------|----------|------------|---------|--------------|
| | Estimate | Std. Error | z value | Pr(> z) |
| MV - C == 0 | -0.02639 | 0.10253 | -0.257 | 1 |
| MVR - C == 0 | 0.02062 | 0.10133 | 0.203 | 1 |
| S - C == 0 | -0.53899 | 0.11866 | -4.543 | 2.78e-05 *** |
| MVR - MV == 0 | 0.04700 | 0.10201 | 0.461 | 1 |
| S - MV == 0 | -0.51261 | 0.11924 | -4.299 | 6.86e-05 *** |
| S - MVR == 0 | -0.55961 | 0.11821 | -4.734 | 1.32e-05 *** |

3. *S* has significantly more individuals than MVR plots.

This pattern was only observed in the predator group (Figure 3). Additionally, though not quite significant it is worth noting that C had near-significantly more individuals than MVR as well (Table 6).

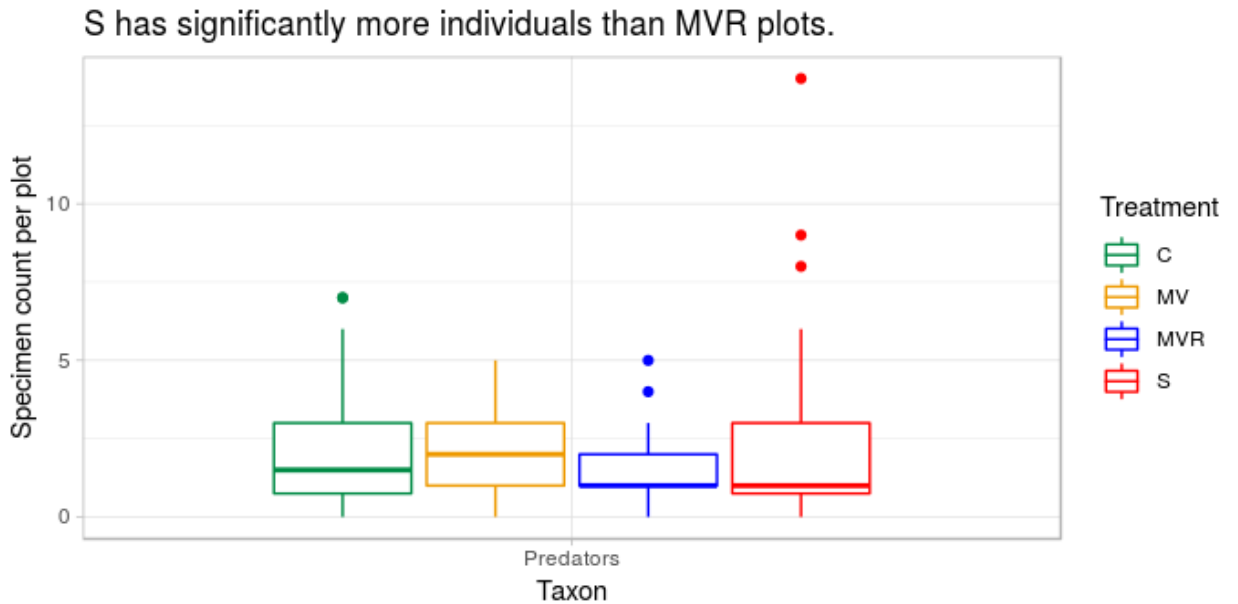


Figure 3. Predators demonstrate lower abundances in plots in which shade treatments were applied than in plots where *M. vimineum* was growing but then experimentally removed.

Table 6. Predator results

| Linear Hypotheses: | | | | |
|--------------------|----------|------------|---------|----------|
| | Estimate | Std. Error | z value | Pr(> z) |
| MV - C == 0 | -0.11279 | 0.16738 | -0.674 | 1.0000 |
| MVR - C == 0 | -0.46734 | 0.18523 | -2.523 | 0.0582 . |
| S - C == 0 | 0.06453 | 0.16003 | 0.403 | 1.0000 |
| MVR - MV == 0 | -0.35454 | 0.18944 | -1.871 | 0.2451 |
| S - MV == 0 | 0.17733 | 0.16489 | 1.075 | 0.8466 |
| S - MVR == 0 | 0.53187 | 0.18299 | 2.907 | 0.0219 * |

Non-metric multidimensional scaling (NMDS)

To explore patterns that may not be visible simply by analysis of individual groups, the species composition of all sample cups was subjected to an NMDS analysis. The NMDS produces a result such that samples with similar compositions are represented as points that are close to one another. Ideally, if overall compositions are affected by the treatments (indicated by color) you would see grouping of points of the same color. After graphing the compositions in an NMDS, the points appeared randomly dispersed and not affected by treatment (Figure 4). That is, being of the same treatment did not appear to lead to similarities in composition for samples receiving that treatment. To verify that points were not arranged group-wise statistically, ovals of standard deviation were mapped. As seen in Figure 4, ovals did not appear to occupy notably separate areas of the graph. To verify a lack of relationship between point distribution and treatment, an **adonis** from R package **vegan** was run on the data. The **adonis** function examines variance using distance matrices and is commonly used in ecological community data in which samples are plotted in accordance with species matrices (Oksanen). The **adonis** attributed point positioning to be .01608 related to treatment with a p-value of .821, indicating that overall composition was almost certainly not affected by treatment.

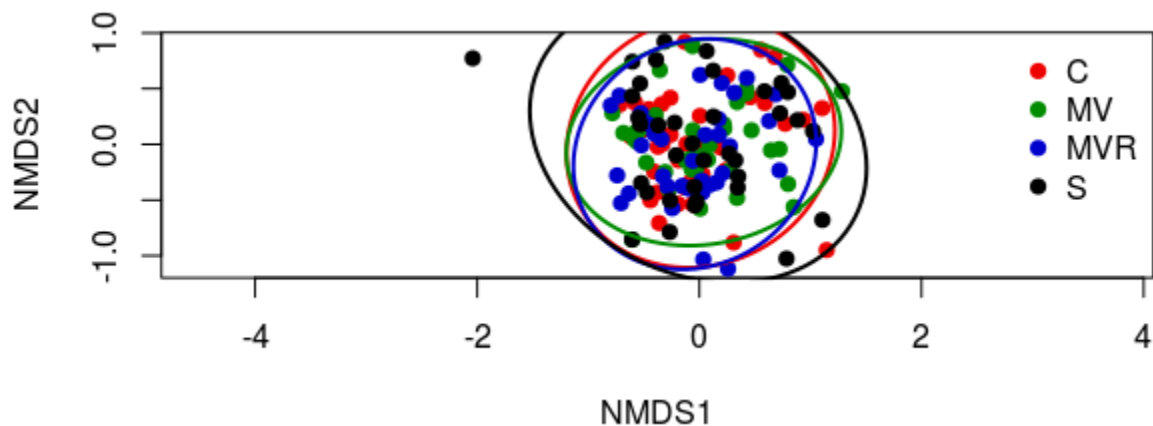


Figure 4. Composition of sample cups was not related to treatments.

Discussion

In theory, since there were 4 groups (so six comparisons: MV-MVR, MV-C, MV-S, MVR-C, MVR-S, S-C) that had the potential of having significantly more, significantly less, or no significant relationship with each of the other groups numerous possible significant variances had the potential of occurring, so it is interesting that we observed only 3 patterns of variance in these 5 significant groups.

Importance of variance pattern 1

As seen in the results, significantly more Spirobolidae and Cosmetidae specimens were caught in plots that were naturally overgrown with *M. vimineum* (MV plots) than in control plots (C, where no *M. vimineum* was growing) and shade plots (S, no *M. vimineum* growing but shaded artificially with shade fabric). However, MVR plot quantities (*M. vimineum* naturally growing but experimentally weeded) were not significantly different than any of the other groups for these taxa. This suggests for both groups that something found in MV plots but not in S or C plots led to a greater number of these taxa. The most immediately obvious quality of MV plots not present in the other two groups is the presence of *M. vimineum* itself. The lack of significance in any MVR relationships does complicate the possible interpretation of this type of variance. Had MVR plots also captured significantly less of these taxa than MV, then all plots without *M. vimineum* growing would have shown the same pattern, which would have suggested that some structural property of *M. vimineum* was at work. Had MVR plots captured significantly more of these two taxa, than S and C did, then we could have concluded that something common to MV and MVR led to the greater quantities, such as soil effects. The absence of any significance in MVR suggests a combination of these mechanisms and/or unknown mechanisms are at play.

Importance of variance pattern 2

Formicidae and omnivores were both caught significantly less in S plots than they were in MV, MVR, and C plots. In this case, results suggest that something about the S plot was less hospitable to Formicidae and omnivores. Although the S plot design was introduced to mimic the effects of shading under *M. vimineum*, the statistically greater number of these groups under MV plots compared to S plots strongly suggests that another mechanism is at play and that the S treatment may not have acted as the ideal mimic of shading effects by *M. vimineum*. One mechanism that this study did not specifically address in its design is that of leaf litter effects. Although the shade cloth placed over the S plots was finely netted and not completely impenetrable, the amount of precipitation that penetrated through the cloth was likely reduced when compared to the natural *M. vimineum* canopy. The shade cloth, on its sturdy frame, would be less prone to giving way under forceful precipitation than bending, windblown grass leaves would be expected to. This consideration is important to the discussion of leaf litter because it is well-established in the literature that the amount of precipitation that accesses the leaf litter layer is significantly related to leaf litter decomposition rates, with increased, more intense precipitation leading to faster decomposition (Austin 2001, Salamanca 2003). Finally, it should also be considered that the more impervious nature of the shade cloth acted as a barrier to further leaf dropping from above, which could also drive change in the leaf litter layer that had implications for the Formicidae and omnivore taxa.

Importance of variance pattern 3

In predators, there was more capture in S plots than in MVR plots. With this alone, potential conclusions could be either that S plots had some factor that was beneficial to predators that was absent or less present in MVR plots, or that MVR plots had some factor that was non-conductive

to predators that was absent in S plots. In order to provide additional clarifying information, the near-significance (.0582) of C plots having more predator individuals than MVR plots should be integrated into this discussion as a possible clue about what mechanisms may be acting. This increased abundance of predators caught in C plots versus MVR plots indicates a greater likelihood that, as considered above, MVR had some factor that was non-conducive to predators. Since MV did not show the same relationships to S and C, this indicates that something that distinguishes MVR plots uniquely from C and S plots, but not necessarily from MV plots may be at work. A commonality of C and S plot design that is not shared by MVR is that there was never any natural growth of *M. vimineum* in C and S plots, and thus there may be some soil-level difference at play. Since *M. vimineum* has been shown to impact soil chemistry (McGrath and Binckley 2009), it may be expected that chemical differences involved in the soil of MVR plots would also be present in MV, but since this study was conducted over 3 weeks period, fluxes in soil chemistry under MVR as a result of removal likely differentiated soil in MV and MVR plots. This is evidenced by the fact that introduction of *M. vimineum* and its removal can cause significant soil changes, notably changes in pH, within one growing season, but can continue to change even after 12 months (McGrath and Binckley 2009, Tekiela and Barney 2015). This indicates that soil changes begin to take place after removal, differentiating MVR soil from MV soil, but not enough to make MVR soil equivalent to uninvaded C plots. Lastly, soil disturbance from the act of removal is another potential player in these relationships (Tekiela and Barney, 2015). While evaluating soil relationships to predators, it is important to note that a group of invertebrates not included in these statistical analyses are members of the microarthropod community, as they primarily fall below 2 mm. Thus, a potential way soil changes could act to affect predators is by changing the availability of predator food sources within the

microinvertebrate community. Not only are microarthropod communities sensitive to soil changes, but multiple microarthropod taxa, notably springtails and mites, are significant dietary sources for higher trophic levels including arachnids and centipedes (Johnston 2000). So, although we can't be sure there were significant differences in microarthropods among treatments, microarthropod predation changes are a plausible way that soil could affect predators.

Individual taxa show varied responses to same mechanistic stimuli

This set of results provides a new perspective about approaching the study of primary trophic level interactions with higher trophic levels. Traditionally, it has been common practice to evaluate invertebrate community measures such as richness and abundance in a more holistic sense, but this approach has led to conflicting results throughout studies, with abundance and insect richness occasionally decreasing and occasionally increasing under *M. vimineum* cover. This study found no significant results for either of these variables and found that invertebrates respond variably to mechanisms associated with *M. vimineum* invasion. Therefore, such a community-wide approach likely varies depending on what taxa of invertebrates are present, with some taxa possibly responding favorably to changes associated with *M. vimineum* invasion, and some taxa reacting negatively, emphasizing the importance of taxon-specific studies.

Exact mechanisms producing the measured changes were less obvious than expected. Instead of the expected patterns of covariance (MV/MVR covariance or MV/S), three other patterns were produced that were less intuitive to make conclusive mechanistic determinations about. If we consider the arguments presented in the introduction about several mechanisms having the opportunity to act on a taxon at once, this lack of discovery of one mechanism acting in each pattern of variance is logical. Since several mechanisms act at once, including possible

mechanisms that weren't being specifically measured, proposing a most active mechanism in each case will not capture the entire ecological network.

Even with this complex network of mechanism effects, an approach that suggests a major mechanism is still very useful because these results would be useful in designing an experiment that examines a specific taxon. For instance, in Formicidae, knowing that this study proposed a major mechanism of leaf litter effects would be helpful in designing an experiment to learn more about Formicidae/invasive plant relationships because it would give the researchers a supported lead on a variable to measure. Additionally, breaking invasive effects down by mechanism allows for a clearer understanding of what aspects of invasives may cause significant changes for certain taxa in a broad variety of other systems.

Study limitations and further research

Four possible ways to adjust this experiment to provide increased clarity were clear.

Firstly, due to initial struggles coding the variable for treatment blocks in R, a 'blocks' measure was not included as a random effect in the full nor reduced mixed effects models. Since isolating random effects reduces the random variance that would have been absorbed by the other variables, it is most likely that not accounting for this variable caused decreased significance rather than increased significance to be observed. Therefore not accounting for this measure would not invalidate the findings of this study. My understanding of R over the course of this study has advanced such that I would now be able to apply a 'block' random effect, and it is my hope to do so. Through adding this measure, I would examine what other significances might result, and how these may add to the larger picture in understanding the significance patterns that were observed.

Secondly, given that leaf litter effects may have played a role in changes that were observed in Formicidae and omnivores, a more direct measure of leaf litter qualities in these groups would have increased the potential for a firmer conclusion, and should certainly be evaluated in future studies as a potential mechanism of invertebrate population change due to invasives.

Thirdly, a fifth type of treatment in which *M. vimineum* was experimentally added may have provided more information, although the exact approach would have been difficult. Experimental addition of *M. vimineum* to plots in the Duke forest, which was the study site, would have had significant ecologically ethical and administrative hurdles that would also be encountered by testing in other natural areas. Additionally, testing for macroinvertebrate changes in a greenhouse would likely be impossible, as research greenhouses are controlled against interference by invertebrates. However, if some way to introduce *M. vimineum* in a controlled setting were used, this plot type would allow us to measure whether there is some aspect of the introduction of *M. vimineum* that causes invertebrate community changes. Differences between MV plots and this theoretical plot could then suggest that possible fluxes happening during introduction, such as changes in soil or moisture, are particularly impactful.

Finally, since four of the five significant groups were found to vary significantly between S and MV plots, it seems highly possible that the shaded plots were producing an effect that was not limited to their shading abilities. One way I could have explored possible effects of the shaded plots in comparison to the other types could have been by monitoring temperature of the plots. While temperature recording devices were placed, the equipment was not successful in capturing accurate data, and this prevented me from being able to evaluate the way that shading altered temperature. This would have been expected to be a primary way that shading would

affect invertebrates. Despite this lack of information to clarify the whole picture, my evaluation that adding a shade cloth may disrupt the access of precipitation to the ground brings to light a potential shade modeling issue that could be improved in the future. Though science has traditionally used shade cloth as a model of shade effects, a plot structure design that mimics shade but is penetrable to precipitation and debris would likely be useful in a variety of potential ecological experiments, and could include a structure over the plot with flaps made out of plastic or some other elastic material among other possibilities.

Overall, this study opens up several potential areas of further interest that would logically follow these findings. It would be useful to see this experiment repeated in a different region or biome to ensure that mechanistic effects are consistent in groups and taxa. In a broader sense, it would also be important to confirm that various taxa continue to respond variably from one another throughout diverse backgrounds, because this would uphold the importance of taxon-specific and mechanistic approaches as an explanation for higher-trophic-level inconsistencies in overall community changes as a result of invasive plants. Additionally, if the experiment was repeated using other invasive plants, and variance among taxa responses to the same invasive-related stimuli was found again, this would provide further support for this argument. Focus on invasive plant effects in other primary producers has yielded important findings, but it is essential that more ecological work is done on invertebrates, as bio-indicators and often one of the most deeply-entwined trophic levels with the primary producers.

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