# Evaluating the effects of Japanese stiltgrass on ground-layer arthropod diversity

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**Introduction**

Invasive species pose an existential threat to native biodiversity all across the United States. Invasive plants are of particular concern, as invasions decrease available habitat and survival of native plant species (Vilà et al. 2011) and can create lasting impacts on multiple trophic levels. Healthy, closed-canopy forests are particularly vulnerable to the establishment of shade-tolerant alien plants (Martin et al. 2009), presenting major challenges to the ecological conservation of old-growth forests and restoration of secondary forests in the United States. These exotics often spread quickly, resulting in novel forest understory habitats and rapidly changing forest communities (Landsman et al. 2020).

Japanese stiltgrass (*Microstegium vimineum*) is a noxious invasive weed native to Asia that invades native forest undergrowth in the US, choking out native plants and preventing further forest regeneration. In invaded areas, *M. vimineum* typically grows in a large, even monoculture, and often outcompetes native plants in low-light areas, reducing plant species richness and native habitat area (Adams and Engelhardt 2009). The shade-tolerant grass spreads quickly across historically heterogeneous forest floors (often a mix of moss, ferns, shrubs, and other forest grasses), potentially leading to long-term effects on native biodiversity (Adams and Engelhardt 2009). Recent studies have found that *M. vimineum* abundance in Tennessee had increased by 50% from 2000-2011, indicating rapidly changing arthropod community structures (Martin et al. 2009). Proximity to forest edges and roadsides heavily contributes to wind dispersal of propagules, which leads to patchy distributions of *M. vimineum*-dominated areas (Huebner 2010). High growth rates in low-light areas can be partially attributed to its C4 photosynthesis pathways, which gives it a light-harvesting advantage over native C3 plants when water availability is not an issue (Warren et al. 2010).

While it is well documented how *M. vimineum* disrupts native herbaceous plants in the US, it is less understood how it affects native arthropod diversity (Simao et al. 2010). Studies tracking herbivore diets have found that some herbivores in invaded areas derive a significant portion of their food from C4 sources (Bradford et al. 2010, Tang et al. 2012), indicating notable herbivory of stiltgrass. It is possible that increases in overall herbaceous biomass in the understory of forests may be preferable for some insect herbivores, providing a dual benefit of food and shelter. Higher density of herbivores could subsidize higher trophic levels such as arthropod predator populations as well (Landsman et al. 2020).

However, the effect of *M. vimineum* on multi-trophic interactions remains in the early stages of study. So far, the limited number of studies conducted on the effects of *M. vimineum* on arthropod communities suggest the effects are highly context-dependent. Some existing studies have shown conflicting results in experimental versus observational approaches. Simao et al. (2010) artificially invaded plots with *M. vimineum* and found that invasion reduced arthropod abundance and richness, particularly in higher trophic levels. Other similar studies tried an observational approach and found opposing results, where arthropod diversity and abundance increased due to stiltgrass invasion in forest understory (Tang et al. 2012, Landsman et al. 2020). Arthropod diversity remains to be a pivotal part of forest ecosystem health in the US, and proper understanding of rapidly changing understory communities will be crucial to guide forest management efforts of *M. vimineum* in the future (Hartshorn 2021).

This study aims to evaluate how invading *M. vimineum* affects arthropod abundance and diversity in southwest Virginia forest understories. Due to its ability to create thick monoculture sheets in shaded forest understories, I hypothesized that *M. vimineum* provides more habitat, herbaceous biomass and ample shelter for arthropod herbivores. Furthermore, I hypothesized that increased herbivore abundance due to *M. vimineum* invasion would have a cascading effect on arthropod predators, as predators will follow the herbivores looking for prey. I therefore predicted that grass-feeding arthropod herbivores and their arthropod predators would be more abundant and diverse in *M. vimineum* than native understory grasses. Moreover, I also predicted that the overall arthropod community composition will differ between native grasses and *M. vimineum*.

## Methods

### *Study species and area:*

This study was conducted in southwest Virginia at the following sites: Pandapas Pond (37.2819° N, 80.4682° W) and Fishburn Forest (37.171132, -80.471872). Both sites are closed-canopy, temperate deciduous forests dominated by hardwoods and native brush. The undergrowth in these forests have been partially invaded by many patches of *M. vimineum*, often not far from trails, streams, or forest edges, where higher wind and human activity can better spread seeds (Huebner 2010). Pandapas Pond is a community area in the Jefferson National Forest with ample human activity, whereas Fishburn Forest is primarily used by the Virginia Tech Forestry Department (Gilboy et al. 2014).

At each site, multiple plots were chosen to represent both native and invasive species. At Pandapas Pond, two invasive plots and three native plots were sampled, whereas at Fishburn Forest there were two invasive and two native. Each 5x5 meter plot was located in the centermost area of a grass patch. Patches smaller than 5x5 meters were not included in the study. Native plots contained a heterogeneous mix of forest grasses (e.g. tall fescue, switchgrass, indian grass) and invasive plots were dominated by *M. vimineum*. The plots were separated by at least 0.1 kilometer. Native sites were found in many locations throughout the understory, whereas invasive sites were found mostly nearby forest roads/trails and streams.

*Procedure:*

Each site was sampled once per week over a period of three weeks in autumn 2021 (October 22, October 27, and November 1). Sampling could not continue past November 1st due to the death of the majority of *M. vimineum* in late autumn. In order to minimize confounding weather variables, both sites were only sampled at midday on warm, dry, and sunny days. At each site, a sweep net was used at each plot to capture arthropods residing in the grass. The sweep nets were aggressively swept through the grass for 90 seconds, with all contents of the net being immediately sealed in a plastic bag. The bag was then placed in the freezer for 24 hours, killing all arthropods for later analysis.

Each arthropod was counted and identified to order, and then to either family/superfamily. Each specimen was also labelled with its relative trophic group (predator, omnivore, or herbivore), determined using the predominant trophic group of species in that family. Arthropods that were best classified as omnivores (e.g. ants) were removed from the analysis (Metcalf 2013), as these were uncommon and did not have enough individuals to accurately be represented. Identifications and determinations of trophic group placement were made using Whitfield and Purcell III (2014).

*Analysis:*

All analyses were completed in R version 4.1.1 (R Core Team 2021). Plot arthropod abundance was calculated by taking the sum of all individuals in each plot, whereas richness was a count of how many different families of arthropods were found in each plot. To compare biodiversity, I used Simpson’s diversity index (Simpson 1949) which was calculated using the vegan package (Oksanen et al. 2020), represented by the formula:

$D = 1-\boldsymbol{\Sigma}(n/ N)^2$.

Data was fitted to generalized linear mixed-effect models using the lme4 package (Bates et al. 2015). Response variables were abundance, richness, and Simpson’s diversity (analyzed separately). Abundance and richness displayed a Poisson distribution, whereas the Simpson’s diversity displayed a normal distribution. Grass type (invasive or native) was included in all linear models as a fixed effect. Site was included as a random effect in all models. To examine whether the arthropod community differed between invasive and native grasses, a non-metric multidimensional scaling (NMDS) model was created using the metaMDS() function in vegan (Oksanen et al. 2020) and tested the difference using a PERMANOVA. Grass type (invasive or native) was used as the predictor variable for the PERMANOVA, and the response variable was the arthropod community. Samples that contained no arthropods were excluded from the data to properly run the analysis.

## Results

In this study, I found a total of 109 arthropods across 6 orders and 18 families/superfamilies. True flies (order Diptera) were the most abundant throughout, and Hemipterans representing families Cercopidae and Cicadellidae were the most abundant of other insect species. Wolf spiders (Order Araneae: Family Araneidae) and ants (Order Hymenoptera: Family Forcididae) were the least abundant, with only one individual found for each throughout all samples. Arthropods in the families Cicadellidae, Cercopidae, Pisauridae, Agelenidae, Asilidae, and Circulionidae were found with >/= 2 individuals on average per plot that they were found in, indicating that those individuals were more likely to be found in larger groups than by themselves.

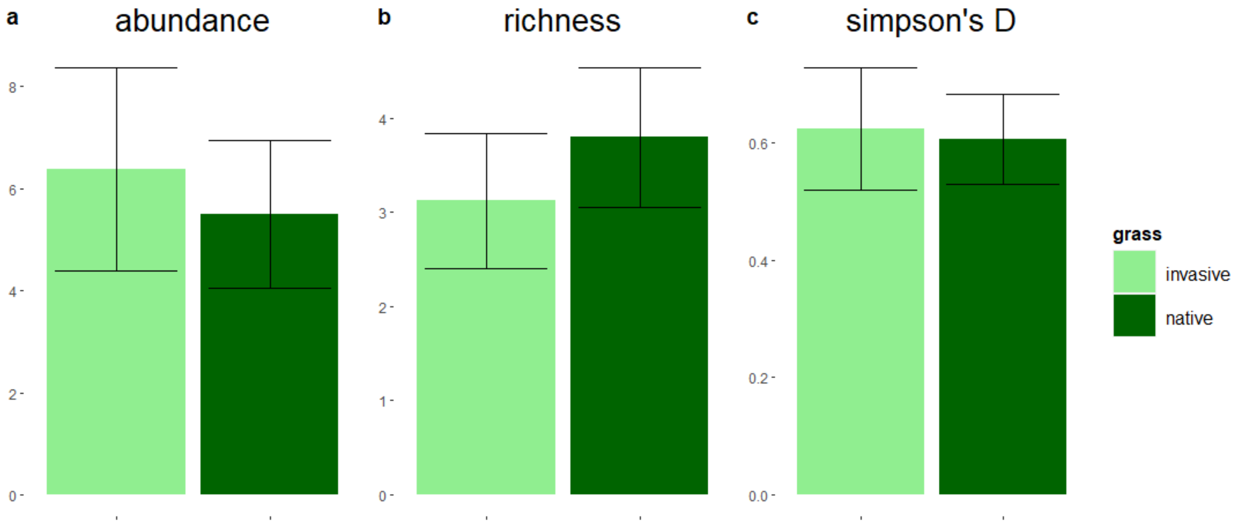

Figure 51: Mean (+/- SE) abundance (a), richness (b), and Simpson's diversity index (c) of arthropod communities found in forest understudy sweep net samples of both invasive and native forest grasses in southwest VA. Invasive grasses were dominated by Japanese stiltgrass (*Microstegium vimineum*), whereas native grasses were a heterogeneous mix of forest grasses. All sampled sites were located in Montgomery County, VA.

Through all analyses, arthropod diversity did not show any evidence of an effect due to grass type. Total arthropod abundance (z= -0.759, p= 0.448), richness (z= 0.759, p= 0.448), and average Simpson's diversity (t= -0.320, p= 0.753) were unaffected by grass type (Fig. 1). Average abundance was slightly greater in invasive sites, whereas average richness was slightly greater in native sites. Average Simpson's diversity was ~0.5 in both grass types. High variance was found in all measurements of diversity.

Average predator abundance was consistent between grass types (z= -0.165, p= 0.869, Fig. 2), whereas average herbivore abundance was about 0.5 higher in invasive grass (z= -0.830, p= 0.407, Fig. 2). Neither predators or herbivores showed any significant effect from grass type, with all averages having high variance. Community structures showed no evidence of a difference between grass types (F=0.956, p=0.512, Fig. 3). While the NMDS showed some clustering of native grasses, high variability in both grass types made it difficult to discern a difference between them.

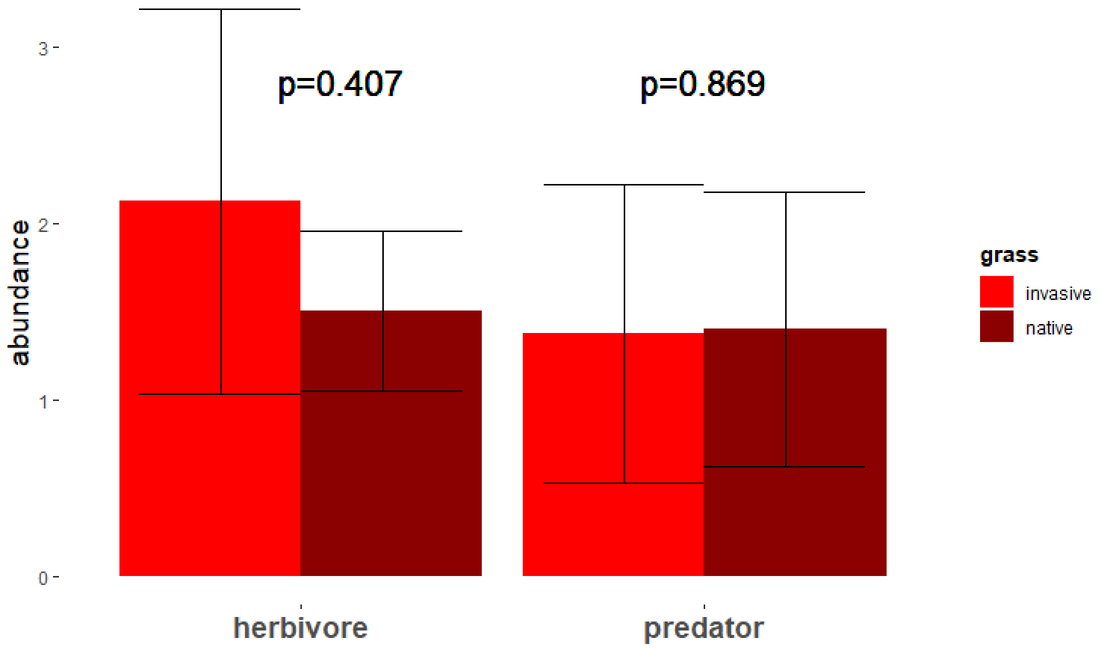

Figure 2: Mean (+/- SE) abundance of arthropod herbivores and predators in a forest understory sweep net study conducted in southwest VA. Predators and herbivores were separated by the predominant trophic state in each family. Invasive grass was dominated by Japanese stiltgrass (*Microstegium vimineum*), whereas native grasses were a heterogeneous mix of forest grasses. All sampled sites were located in Montgomery County, VA.

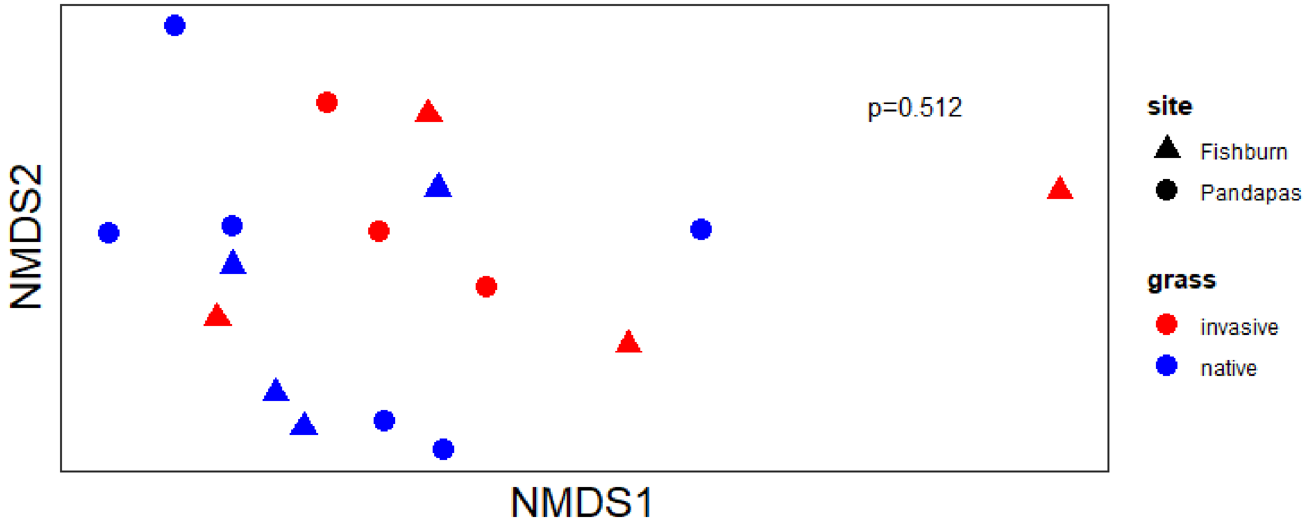

Figure 3: Non-metric multidimensional scaling (NMDS) model of arthropod communities in a forest sweep net study in southwest Virginia. Both grass type (invasive/native) and site (Fishburn Forest/Pandapas Pond) are visualized in the model. Each point represents a single sweep net sample. Invasive grass plots were dominated by Japanese stiltgrass (*Microstegium vimineum*), whereas native sites were a heterogeneous mix of forest grasses. Sites are both located in Montgomery County, VA.

## Discussion

I collected arthropod samples in a directed study to discover the relationship between arthropod composition and *M. vimineum* in invaded understories. Of all arthropods, I found that true flies (Order Diptera) and true bugs (Order Hemiptera) were the most abundant orders across all plots. Overall, grass type did not cause any difference in diversity, as arthropod abundance, richness, and Simpson's diversity all showed no significant evidence of an effect. Arthropod herbivores were found to be slightly more abundant in invasive grasses than native grasses, though due to high variation that cause was not substantiated (Fig. 2). Overall community structure was also not different between grass types or sites (Fig. 3). Some native grasses seemed to show some similarity between each other, though high variance makes it difficult to clearly distinguish.

The results did not provide evidence that *M. vimineum*-invaded understories provided a better habitat for arthropod herbivores or their predators. High variation of samples indicate that arthropods did not have a clear preference for native or invasive grasses in these southwest Virginia forests (Fig. 1). In fact, this study suggests there may be a lack of a significant community impact by *M. vimineum*. Previous research has found evidence that *M. vimineum* has multi-trophic effects on arthropod communities, where increased abundance and diversity can be seen evenly throughout numerous different sites (Tang et al. 2012, Landsman et al. 2020). Opposing results from different studies with the same focus show a decrease in arthropod abundance and diversity (Simao et al. 2010). Nonetheless, the ecological complexity of these systems may be more complicated than previously thought, which is demonstrated by the lack of a consistent narrative throughout literature. Likewise, a lack of significant and directional results in my study may be indicative of site-specificity, which could be due to several confounding variables.

Non-unidirectional context-dependency is one possible explanation, as is the case with many generalist invasive species (Vilà et al. 2011). Untested abiotic components such as grass moisture and shade could have caused a response by arthropods in their choice of habitat, which can vary within a single system. Ecological heterogeneity due to different conservation and disturbance regimes of forests (e.g. agriculture) may also be notable factors in arthropod community structures in *M. vimineum* invaded forests (Martin et al. 2009). Arthropod distributions can vary greatly across regions and biomes (Gossner et al. 2014) compared the generalist nature of *M. vimineum*, so the effect of stiltgrass on arthropod communities may be partially dependent on the specific habitat in which it is sampled. Nonetheless, the substantial difference in vegetation was still enough to expect an effect on community structure despite the influence of other factors.

The native and invaded sites available around Blacksburg may have differed in other ways, as *M. vimineum* patches tended to conglomerate around streams, roads and trails (see Huebner 2010). Potentially, the lack of variation by grass type in my results could be explained by temporal factors (Adams and Engelhardt 2009, but see Tang et al. 2012), which could have caused some bias in the results. A sampling period cut short by the annual death of *M. vimineum* may have only provided an isolated snapshot of the community structure. *M. vimineum* seemed to die off quickly after seeding in September, leaving a small window for autumn sampling. Moreover, leaf litter accumulated throughout the sampling period, and inhabiting arthropods that are less connected with *M. vimineum* may have affected the resulting arthropod communities that were sampled. Perhaps looking at the impacts of *M. vimineum* over multiple seasons (like Simao et al. 2010) in a similar observational study could provide a more complete picture of the relationship. Using other sampling methods such as pitfall traps and sticky traps in addition to sweep netting may also help to eliminate sampling bias.

Overall, the impacts of *M. vimineum* remain somewhat ambiguous, highlighting a need for more thorough understanding of habitat-specific and multitrophic effects (Sanders et al. 2004). Though I found no evidence of an effect of *M. vimineum* on arthropod communities, knowledge of the arthropods that live in the grass could still be of use to land managers. Furthermore, an understanding of arthropod communities in a particular location is important for management purposes, as the implications of using chemical or physical methods to control the spread of *M. vimineum* likely has effects on the arthropod community residing in the grass. Future research on this topic would be better focused on sampling arthropods in different *M. vimineum*-invaded habitats on a more regional scale to identify dominant arthropods living in the stiltgrass in each area. Land-managers looking to control *M. vimineum* invasions could then tailor their control techniques to their area and try to lessen the impact of intervention on existing arthropod communities. Though the results from this study suggest that *M. vimineum* impacts on arthropod communities are indistinguishable in southwest Virginia, it emphasizes that the consequences of invasion can be highly variable across different systems.

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# Tables

Table 1: Abundances and site appearances of each family/superfamily of arthropod in a forest understory sweep net study conducted in southwest VA. All counts were collected in Montgomery County, VA.

| Order | Family/ Superfamily | Total Abundance | # of appearances | Average abundance* |
|---|---|---|---|---|
| Orthoptera | Caelifera | 3 | 3 | 1 |
| Hemiptera | Cicadellidae | 16 | 7 | 2.29 |
| Hemiptera | Reduviidae | 3 | 3 | 1 |
| Hemiptera | Cercopidae | 11 | 5 | 2.2 |
| Hemiptera | Pentatomidae | 2 | 2 | 1 |
| Hemiptera | Aphidoidea | 3 | 2 | 1.5 |
| Araneae | Pisauridae | 10 | 5 | 2 |
| Araneae | Agelenidae | 9 | 6 | 1.5 |
| Araneae | Oxyopidae | 3 | 1 | 3 |
| Araneae | Araneidae | 4 | 2 | 2 |
| Araneae | Salticidae | 2 | 2 | 1 |
| Araneae | Lycosidae | 1 | 1 | 1 |
| Diptera | Asilidae | 12 | 6 | 2 |
| Diptera | Sciaroidea | 18 | 11 | 1.64 |
| Coleoptera | Curculionidae | 4 | 2 | 2 |
| Coleoptera | Carabidae | 2 | 2 | 1 |
| Coleoptera | Chrysomelidae | 2 | 2 | 1 |
| Hymenoptera | Forcididae | 1 | 1 | 1 |

*Average abundance: calculated by taking the average number of arthropods at all 5x5 meter sweep net plots where they made an appearance (e.g. higher “average abundance” means that sites where they are found are more likely to have multiple, etc.)