

Toward a More Integrative Approach to Quantifying the Ecological Impacts of
Invasive Plants

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Plant Pathology, Physiology, and Weed Science

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March 22nd, 2016
Blacksburg, Virginia

Keywords: Ecological impact, invasive species, Japanese stiltgrass, *Microstegium vimineum*

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ABSTRACT

“I do not cherish the utopian dream that we shall ever be rid of weeds, but there are some kinds that ought to receive the most earnest attention”

Aven Nelson 1896

Invasive species are reported as one of the top current and future concerns for the health and functioning of native ecosystems. In response, identification of invasive plant impacts are one of the top most studied aspects of plant invasions. Yet we still know very little about invasive plant impacts, and many land managers remove invasive plants not because of known negative impacts, but because of the general negative connotation associated with invasions. Here, I develop and utilize integrative methods to more holistically measure the ecological impacts of invasive plants. I develop a meta-analysis of current invasive plant impact literature, I integrate independent ecosystem metrics into holistic measures of total ecological impact, I examine the potential for legacy and temporal effects in newly established and recently managed invasions, and I measure ecological impact of co-invaded ecosystems. Through these studies, I find that magnitude, and not direction, of impact better represents actual ecosystem changes when evaluating invasions holistically. I also find invasive plant management may not only fail to remove long term legacy effects, but may in some cases further negatively impact the plant community. Finally, I find that co-invaded systems are not intrinsically worse off than single invaded systems. Collectively, these studies help to better our understanding of the impacts of invasive plants and their implications for management, and show that simple attempts at eradication may not always be desirable.

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GENERAL AUDIENCE ABSTRACT

Humanity is dependent on healthy and functioning ecosystems to protect human health and meet our basic needs. Ecosystem services include clean air, water, and soil by detoxifying and decomposing waste, pollination of nearly all the crops we eat, and moderation of weather extremes. Sadly, we have degraded most ecosystems through the process of global change, which encompasses climate change, pollution, disease, habitat fragmentation, and importantly, invasive species. Invasive species are typically non-native species that are transported to new areas, often by humans, and become incredibly successful at the expense of native species. In addition to endangering native species, invasive species reduce the health of ecosystems which in return reduces the services they provide. Therefore, billions of dollars in the U.S. alone are spent removing invasive species. To determine the most economical use of limited resources to manage these species, scientists measure the “ecological impact” that each species imposes on the environment. Determining what needs to be measured and how should it be measured is not straightforward. Here, I create a better understanding of how scientists currently measure and interpret invasive plant impacts and suggest new ways to better understand ecological impact that looks at ecosystems as a whole, not piecemeal. Through this framework I find that although we can physically remove invasive plants from the environment, that does not mean we remove all their impacts. Finally, I find ecosystems that are invaded by invasive plants that are physically similar may be less impacted than when very different (i.e., grass and shrub) invaders co-occur. These studies will help to better understand the impacts of invasive plants and importantly

suggest that simply removing the invasive plant from the environment may not always lead to healthier ecosystems.

Dedication/Acknowledgements:

I thank Dr. Jacob N. Barney for acting as both my mentor and advisor throughout my graduate program. Dr. Barney helped develop me into a scientist that I never could have imagined to be prior to this program. Thank you.

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Chapter 1: Different methods yield dissimilar conclusions of invasive plant impacts: a meta-analysis

Abstract:

Various methods used to quantify ecological impacts of invasive plants differ in their assumptions and execution, which may yield dissimilar impact estimates. We performed a meta-analysis on 547 studies to test how methodology (observational comparisons and invader removals), invader cover, and removal period influenced impact estimates. Observational comparisons showed no overall invader impact, while invader removals showed a negative effect. We performed a second meta-analysis excluding impact direction (i.e., invasion increased or decreased value) to identify potential masking resulting from integration across diverse species. When only considering magnitude (i.e., size of change), both methods yielded similar impacts. Additionally, impact magnitude increased with invader cover and the length of removal. Overall, conclusions about the effects of invasive plants vary between methods when considering impact magnitude and direction, but are similar when focusing on magnitude, which best represents the broad changes resulting from invasion.

Introduction:

There has been a recent spotlight on identifying the impacts of invasive plants on ecosystem structure and function to better understand their role in global change. These ecological impacts include effects on plant (Hejda et al. 2009, Vilà et al. 2011), animal (Litt et al. 2014), and soil microbial communities (Allison et al. 2006), biogeochemistry (Wang et al. 2015), and ecosystem function (Charles and Dukes 2007). In response to the exponential growth in invasive impact studies (Lowry et al. 2013), there has been vigorous discussion on how and why researchers choose the species and impacts they study, as large biases exist (Hulme et al. 2013, Jeschke et al. 2014). In spite of these limitations, several meta-analyses have identified some general trends of invasive plant impacts (Vilà et al. 2011, Pyšek et al. 2012), though tremendous variation exists when aggregating such varied invader life forms across the systems they invade. However, the methods in which ecologists use to come to these conclusions are varied and not standardized.

This lack of a common approach for quantifying invasive plant impacts may have important consequences i) for our ability to identify impacts accurately, ii) it impedes our ability to directly compare impacts across species, sites, and time, and iii) remains an overlooked, yet possibly important, source of variation in existing meta-analyses of invasive plant impacts (Barney et al. 2013; Kumschick et al. 2015b). Invasive plant impacts cannot be determined by only studying an invaded landscape. Instead, impacts are estimated as the difference between the invaded landscape and a reference/control site. Unfortunately, a variety of “references” have been used to estimate invasive plant

impacts, which vary in the assumptions and execution. Despite the acknowledgement of the consequences of using several methods to estimate impact (Kumschick et al. 2015b), no meta-analyses to date have considered the potential implications of study methodology on the estimates and conclusions of invasive plant impacts (Liao et al. 2008, Vilà et al. 2011, Pyšek et al. 2012). We addressed these major limitations by using a meta-analysis to answer the question; do different methods yield different impact results?

Three methods are used to estimate the impacts of invasive plants, which vary in their assumptions and execution—important differences that may result in dissimilar impact estimates. The *observational* method compares the invaded site to a nearby uninvaded reference site, which is an area that is deemed similar in all characteristics except for the absence of the invader. It is assumed that the uninvaded site represents the state of the invaded patch before the invasion (Barney et al. 2015), and thus any measured differences are attributed to the invader. As White & Walker (1997) eluded in the context of restoration; “among the problems of individual reference sites is the difficulty of finding or proving a close match in all relevant ecological dimensions.” Despite the assumptions and shortcomings of the observational method, it remains one of the most common and robust methods for estimating impacts (Barney et al. 2015, Kumschick et al. 2015b).

In some cases, simply finding a nearby ecologically appropriate uninvaded area is difficult, especially in older invasions, which may have colonized all invadable patches. In these cases, an uninvaded reference site may not be available or feasible—necessitating

an alternative approach. The *removal* method compares the invasion to patches within the invasion where the target invader has been selectively removed (i.e., removal reference). This has the advantage of not requiring paired invaded and uninvaded sites. Unfortunately, removal of an invasive plant may “not lead to the recovery of valued ecosystem properties or the successional trajectory toward a more desired condition... In these situations, altered community and ecosystem properties may be a legacy of the past plant invader” (Corbin and D’Antonio 2012). These so-called legacy effects exist when certain ecosystem characteristics do not immediately return to their uninvaded state, which have been documented in soil microbial composition (Belnap et al. 2005) and soil nutrients (Marchante et al. 2008), but not always in plant community composition (Tekiel and Barney 2015). Additionally, the act of removing an invasive species is itself a disturbance, the magnitude of which varies among methods (i.e., cutting vs pulling), which can complicate parsing the effect of the invader from the removal itself. Again, despite these limitations, this technique is also commonly used to estimate invader impacts.

The third method, referred to as an invader *addition*, is the creation of a “synthetic invasion” by adding the invasive species of interest to an uninvaded area, which is then compared to the nearby uninvaded area. Similar to a removal reference site, temporal issues exist in attempting to quantify impacts in synthetic invasions. Just as legacy effects can exist following invader removal, the magnitude of impacts of an invasive plant can change with time since invasion (Dostál et al. 2013, Aicarella and Ankievicz 2015). In other words, for this method, when has sufficient time passed for the synthetic invasion to be considered established and therefore representative of a “natural” invasion? In

addition to logistical uncertainties, ethical questions must be considered when adding an invasive plant into an area not previously established (Minteer and Collins 2010). Our primary objective was to compare the impact estimates among these three methods using a meta-analysis.

Additionally, invasive plants comprise an enormous diversity of life forms and physiologies, and invade nearly every biome on Earth. Therefore, it is not surprising to find large variation and often no overall impact in the search for general trends across this species and site diversity (e.g., Vila et al. 2011). Currently, meta-analyses consider both impact magnitude (i.e., size of change) and direction (i.e., invasion increased or decreased value), the latter of which may mask overall trends as species may negate each other. For example, if one invader increases soil organic carbon by +X units, and another decreases soil organic carbon by -X units; both species significantly changed soil carbon pools, but in opposite directions. These impacts would be “canceled out” when integrating across species. We have argued elsewhere (Barney et al. 2013) that when integrating across diverse metrics or species, a focus on impact magnitude best represents the conservation concern posed by invasive plants—are they changing native systems—that would otherwise be obfuscated with impact direction. Therefore, our second objective was to identify whether the experimental methods are similar or not if only impact magnitude is considered.

Methods:

Data were gathered using the web based scholarly publication search engines ISI Web of Science (<https://apps.webofknowledge.com>) and Google Scholar

(<https://scholar.google.com>) in April 2015. We used the search term: *invas** AND “plant” AND (“removal” OR “manipulate” OR “effect” OR “impact” OR “restoration” OR “management”). This search term returned 8,256 results in Web of Science and 2,180 results in Google Scholar. Results were sorted by relevance in Web of Science and Google Scholar and then articles were systematically screened for experimental designs that recorded quantitative measures of impact and included at least one of the following study methods: 1) observationally compared invaded and uninvaded plots, 2) a removal treatment within the invasion compared to surrounding invasion, 3) an additive treatment where the species of interest was introduced and compared to an uninvaded area, or 4) any combination of these methods. Purely modeling studies and studies that did not expressly make an invaded-reference comparison were not considered. Because of the volume of articles returned by the broad search terms used, when literature no longer included more than one key word, the literature search was halted. The literature cited section of each article was also scanned for any additional articles with relevant methodologies. Only primary literature was considered in the final database, but literature cited in reviews, grey literature, and meta-analyses were also scanned for additional primary literature for inclusion.

This search resulted in 112 uninvaded, 62 removal, and 8 addition articles, 9 of which included both uninvaded and removal methods. Due to the low number of articles using the addition methodology, we did not include them in further analyses. However, this also suggests that addition studies are relatively rare (4% of studies). When multiple impact metrics were measured (e.g., plant biodiversity, soil pH, plant richness) within a single study, the article was further divided into individual tests or metrics and each test

was considered independent. A total of 299 observational and 208 removal metrics were recorded from the 174 studies. The number of tests per article has not been found to influence results in previous analyses and can be treated independently (Vilà et al. 2011), therefore, we considered each test to be independent. Our database was smaller than Vilà *et al.* (2011) due to our more stringent search criteria to test specific clearly defined methodologies.

For each test the response means, standard errors/deviations, and replications per treatment were recorded directly from the text or tables of the article when possible. In cases where data could not be obtained directly, either the corresponding author was contacted requesting the data or software (Datathief; Datathief.org) was used to extract values from figures. In the case of the same test being performed over time or over a range of invader abundance (i.e., cover or density), the difference between longest time or the highest invader abundance was used. Additionally, where available, data were collected on study length, ecosystem type (e.g., forest, grassland), invader life form, and response metric type (e.g., animal, soil nutrient pool) to use as response variables and covariates during analyses. For removal studies, removal type (e.g., herbicide, hand pulling) was not considered because replication within each method was small. Chi-squared tests were performed on contingency tables of methodology type with either ecosystem type, response type, or life form to determine if distributions within methodology type were similar.

As with other ecological meta-analyses (e.g., Vilà *et al.* 2011) we calculated Hedges' *d*, which is a measure of effect size that is relatively robust at small replication

size, which is frequently the case with ecological studies. We used Hedges' d with invaded and uninvaded or removal impact metric values to compute a difference similar to standard statistical tests. However, Hedges' d normalizes these values to the standard deviation of the study to make studies comparable (Rosenberg et al. 2000). Positive Hedges' d means the invaded impact metric value is greater than the reference value, zero mean there is no difference, and negative values mean the invaded impact metric value was smaller than the reference value. To address our second objective that focuses on impact magnitude, we also calculated an absolute value of Hedges' d ($|Hedge's\ d|$). Because this study combines many different impact metrics, the meaning of directionality can be abstruse and largely subjective (e.g., negative pH and positive diversity). Therefore, an absolute value transformation removes directionality and allows for an unbiased assessment of the magnitudes of the difference between treatments.

Random effect models were used to allow variation of effect sizes among studies, and due to its robustness when using non-normal data, DerSimonian-Laird estimator was used at a 95% confidence interval for meta-analyses (Kontopantelis and Reeves 2012). We performed a meta-regression for Hedges' d and $|Hedges' d|$ using only study methodology; or study methodology, ecosystem, life form, and response type to test if study methodology was a significant factor in describing impact. Additionally, a subgroup analysis was performed for each separate categorization (i.e., ecosystem, response type, life form) on study methodology to test if study methodologies were different within categories. However, only dune (n=51), grassland (n=109), forest (n=228), and riparian (n=78) had adequate replication, therefore only they were included in the ecosystem subgroup analysis.

Plant invader cover has also been shown to influence the magnitude of impact for some species (e.g., Jäger *et al.* 2009; Greene & Blossey 2011). Therefore, meta-regression was also performed on |Hedges' d | for invader cover level of studies that explicitly listed cover ($n = 278$). As previously discussed, time since removal can also be important due to legacy effects of the invasive plant, therefore a meta-regression of number of years with the invader removed for removal studies ($n = 208$) was performed. Only |Hedges' d | was used for these analyses because increase in magnitude of responses over each variable could be masked by directionality of responses in Hedges' d . In both cases, ecosystem, life form, and response metric type were included as covariates. All meta-analyses were performed in OpenMEE (Dietz *et al.* 2015).

Results:

Across all studies, the observational methodology showed that the invasion was not different than the uninvaded site (i.e., the CI of the effect size overlapped zero). However, removal studies did show a significant negative impact from invasion (Table 1 and Figure 1). Importantly, the observational and removal methods were also different from each other (Figure 1). In other words, the methodology used gave significantly different outcomes. However, when the effect was transformed (|Hedge's d |) to remove the effect of directionality, the methodologies resulted in similar effect sizes, both of which showed that the invasion was different than the reference (Table 1 and Figure 1) and that invasive plants impact the invaded systems. The sub-group analysis that accounted for ecosystem, response type, and invader life form, explanatory power

increased and each of the sub-group variables was statistically significant but study methodology no longer was (Table 1).

Contingency tables of study methodology with ecosystem type, invader life form, and response metric type all had significant chi-square tests, meaning the distribution of each sub-group within study methodology was different ($p = <0.0001, 0.0144, <0.0001$ respectively; Figure 2).

The effect of study methodology varied strongly among ecosystems though with no consistent trend (Figure 3a). The effect size varied between study methodologies in riparian and grassland ecosystems, but in opposite directions (Figure 3a). When $|Hedge's d|$, the study methodologies were only different in the riparian ecosystem where the observational method had a higher effect size than removal. The only difference between the methodologies for life form was in grasses, where removals had a negative effect size while the observational method was not different than zero (Figure 3b). Regardless of methodology, for most life forms the confidence interval (CI) of the mean effect size overlapped zero, except shrub observational and grass removal. However, when absolute-transformed, all life forms had large effect sizes, and methodologies only varied among trees where tree removal had a larger effect size (Figure 3b).

Effect sizes for response types varied widely in magnitude and direction, with differences between study methods in microbial and ecosystem impact metrics (Figure 3c). Effect CIs were different than zero more often for observational than removal, and were negative for plant, ecosystem, and animal response types (Figure 3c). When transformed, the effect size was similar for both methods within response types.

The magnitude of the transformed effect sizes was moderately positively correlated with removal period ($p = 0.0640$; Figure 4a), and strongly correlated with invader abundance/cover ($p = 0.0194$; Figure 4b).

Discussion:

Across all studies, the overall magnitude of the ecological impact of the target invasive plant varied depending on the method used to estimate that impact. In fact, our results suggest that observational studies show no impact from invasive plants, while removal studies show that invasive plants have a negative effect. Thus, methodology strongly affects the conclusion of whether the target invader is impacting the native ecosystem.

Although our meta-analysis found that removal studies estimate a net negative impact while observational studies show no difference, this conclusion is not corroborated by individual studies that used both observational and removal methodology. Of the nine studies that used both methodologies, six found that the removal methodology had a *smaller* impact magnitude than observational, while the rest found no difference. However, it is not surprising that individual studies show different trends than meta-analyses as meta-analyses must integrate over many different systems with different invaders with different impacts measured.

The differences identified between methods may be driven by, and similarly confounded by the variety of species and habitats studied. The effect of methodology on invasive plant impacts was strongly dependent on the ecosystem in which the study was

conducted, the invader life form, and the identity of the impact metric (e.g., soil pH, microbial community, native plant diversity, etc). This undoubtedly reflects the wide variety of systems, species, and metrics that have been studied. In fact, Hulme et al. (2013) found the systems, species, and metrics that have been studied for impacts to date to be very unevenly distributed. There are few well studied species, systems, and metrics, while the majority are poorly understood. Therefore, why would we expect a common trend in invasive plant impacts across species as wide ranging as sedges to trees invading systems from deserts to alpine terraces? In fact, Vila et al. (2012) also found large variation and very few trends in invasive plant impacts aside from those imposed by nitrogen-fixing species.

Despite the large variation among species and systems, our subgroup analyses within each covariate identified a few areas where impact studies may be more susceptible to study methodology (i.e., different methods give different impact results). For example, riparian systems and invasive trees showed different impact magnitudes and directions between the methods. As was previously discussed, that act of removing the invader is itself a disturbance (Skurski et al. 2013). Trees are often difficult to remove and account for a large proportion of total biomass. Therefore, the larger impact magnitude in tree removal studies could have resulted from the removal itself, and not the presence of the invasive tree—though this would need to be empirically tested. Riparian ecosystems also showed variation in study methodology: observational studies showed a higher impact magnitude than studies using the removal methodology. This could be attributed to either lag effects reducing the magnitude of impact from removal studies, or

an inappropriate selection of observational reference sites, though it is unclear why riparian systems are unique in their response to removals.

Among the impact metrics recorded, plant and animal parameters were consistently negative, and microbial parameters were positive for Hedges' *d*. This corroborates what Vilà *et al.* (2011) found, further suggesting these responses are globally similar in directionality across invasive plant species—invasive plants reduce plant and animal community richness and biodiversity. Additionally, soil metrics had an overall positive increase (in observational studies only) due to invasion as Ehrenfeld (2010) also found, though Vila *et al.* (2011) found wide variation in magnitude and direction among individual soil metrics. Some biogeochemical processes and litter dynamics have been found to be strongly species-specific (Tamura and Tharayil 2014).

The answer to our primary question of whether different methods yield different impact results is yes, the methods are not equivalent, for overall impact integrated across all species, sites, and impact metrics. However, the differences are more subtle, though still remain, when decomposed within invader life forms, ecosystems, and metrics. Importantly, these methodological differences could alter conclusions regarding whether invasive plants are the source of important ecological changes. However, our second analysis that focused on impact magnitude indicated a different picture.

Firstly, in some cases the direction of change is important to the interpretation of the impact of an invader, such as when specific hypotheses regarding the effects of an invader on particular ecosystem properties are of interest (Hulme *et al.* 2014). Certainly the literature is full of examples of the effects of individual species on individual

ecosystem properties (Hulme et al. 2013), which presents a series of problems of their own (Barney et al. 2013). However, because our analysis broadly considers several metrics used to determine impact, the meaning of directionality is difficult to interpret (Pyšek et al. 2012). For example, if pH is higher in the invasion is this ‘worse’ or ‘better’ than an equivalent decrease in pH? Increased soil nitrogen due to invasion may seem like a beneficial effect, but what about in systems where the likelihood of invasion increases with increased nutrient availability (Funk and Vitousek 2007)? Aside from specific impact-metric hypotheses, researchers are concerned with, and land managers often manage invasive species to mitigate their collective impact to the system, regardless of the direction of change (Barney et al. 2015). Thus, we argue that when a multi-impact criterion is of interest to capture broader invasive plant effects, or when looking for meta-effects across species and systems, that impact magnitude should be the focus. Certainly, our analysis highlights the dramatic effect of directionality on the conclusion of meta-effects.

Directionality presents challenges in interpreting invader impacts for single species across multiple criteria, but especially when comparing or aggregating across several species to identify “meta effects”. In cases where different invaders have similar changes in magnitude, but opposite directional effects, the mean would suggest no impact. Individually we would conclude that both species are having impact; however, calculating traditional meta-analysis effect sizes would result in a net of no change, and we would conclude that invasive plants have no impact. This conclusion may lead to the erroneous decision to not manage either species, despite their quantified impacts. It does however, allow us to conclude that invasive plants do not universally change soil carbon

in one direction, which remains important. Thus, there are individual and idiosyncratic changes that are important, which may simultaneously obfuscate broader conclusions about impact. Thus, our transformed effect size, $|\text{Hedge's } d|$, represents the average impact magnitude of invasive plants (Barney et al. 2013).

While most studies do not investigate the relationship between invader abundance (i.e., cover, density) and impact (Barney et al. 2013), some have shown a linear increase in magnitude with grass (Olsson et al. 2012), forb (Hulme and Bremner 2005), shrub (Gooden et al. 2009), and tree (Ruwanza et al. 2013) cover. We have also shown that abundance-impact relationships can be non-linear (Tekiela and Barney 2015), and others have shown more complex relationships (Thiele et al. 2011). Here we show that across all species, systems, and metrics, that impact magnitude increases with invader abundance. This supports the calls for impact studies to explicitly account for invader abundance (Barney et al. 2015). Elucidation of abundance-impact relationships has the added conservation benefit of identification of possible management thresholds, which could aid management prioritization.

We also saw an increase in impact magnitude with increasing time since initial invasive plant removal. This corroborates the hypothesis that legacy effects can exist following invasive plant removal (Corbin and D'Antonio 2012), or that sufficient time is required to recover from the removal disturbance itself. Our results suggest that on average, legacy effects exist in removal studies, and should therefore be carefully considered prior to performing this type of study and in the interpretation of results.

As we continue to understand and catalogue the ecological impacts of invasive plants, we must pay careful attention to the chosen method, which may affect the magnitude, direction, and ability to detect impacts depending on the invasive species and system invaded. When looking for meta-effects of the ecological consequences of invasive species, directionality of impacts should be carefully considered before aggregating across species, ecosystems, and response metrics. Additionally, we have demonstrated a meta-effect of invader abundance on impact magnitude—reinforcing the need to explicitly account for invader abundance. Traditional and magnitude-focused meta-analyses elucidate important trends in invasive plant impacts, both of which add to our understanding and search for broad trends. Lastly, due to the wide variation among studies, we support the need for globally coordinated experiments (e.g., GIIN in Barney et al. 2015) that are designed to reduce variation and increase statistical power to aid in our search for invader meta-effects.

Acknowledgements: We thank Dr. Ryan D. Zimmerman for assistance on specific meta-analytical techniques and Dr. Daniel Z. Atwater for his comments on the manuscript.

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Table 1. Statistical results of meta-regressions showing which factors (i.e., study methodology, ecosystem, life-form, and response metric type, significantly influence for Hedges' d and |Hedges' d |.

Model		R^2	df	Q	p-value
Hedge's d					
Study methodology		0.052			
	Study methodology		1	4.58	0.0323
Study methodology+ Ecosystem+Life form+ Response metric type		0.2044			
	Study methodology		1	0.80	0.3720
	Ecosystem		12	41.92	<0.0001
	Life form		5	13.27	0.0210
	Response metric type		4	71.99	<0.0001
 Hedge's d 					
Study methodology		0.000			
	Study methodology		1	0.050	0.8230
Study methodology+ Ecosystem+Life form+ Response metric type		0.1164			
	Study methodology		1	2.21	0.137
	Ecosystem		12	45.60	<0.0001
	Life form		5	8.03	0.1540
	Response metric type		4	7.61	0.1070

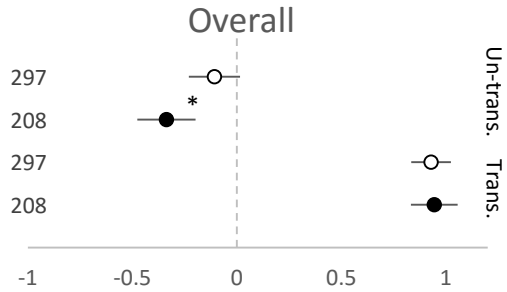


Figure 1. The overall effect of methodology type on both Hedges' d (H) and absolute value Hedges' d ($|H|$). Unfilled circles are observational studies and filled circles are removal studies with 95% confidence intervals. Stars designate significant differences and replications are listed left of data. Negative points represent a negative difference, positive points represent a positive difference, and points intersecting 0 represent no difference.

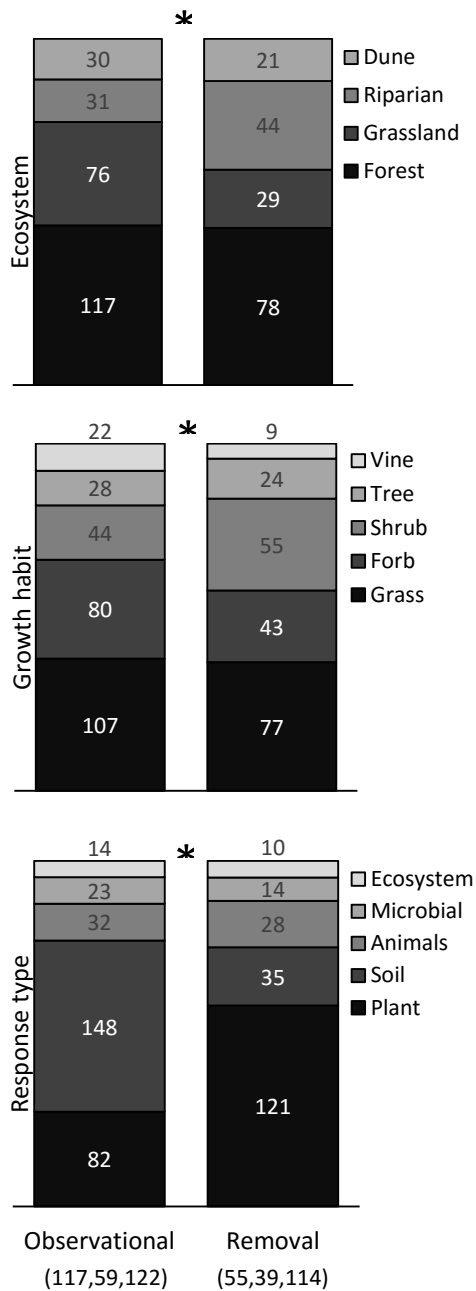


Figure 2. Proportional distribution of studies within each categorization for each study methodology. Numbers within or above bars denote number of studies in each category. Parenthetical numbers denote the total number of negative, null, and positive, studies respectively within each methodology. Asterisk signifies significant Chi-squared tests between methodology types.

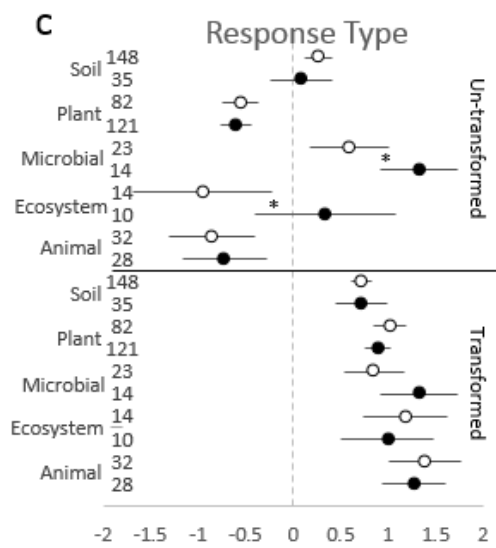
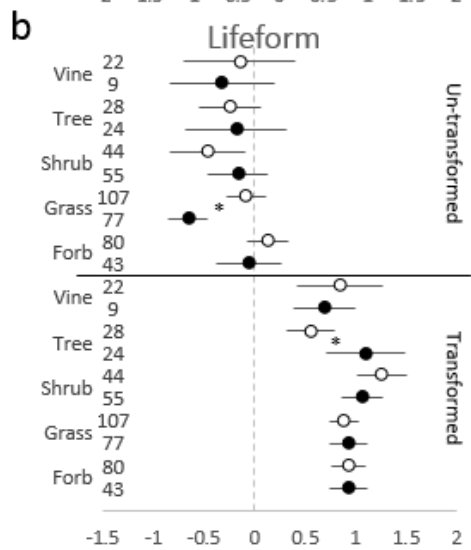
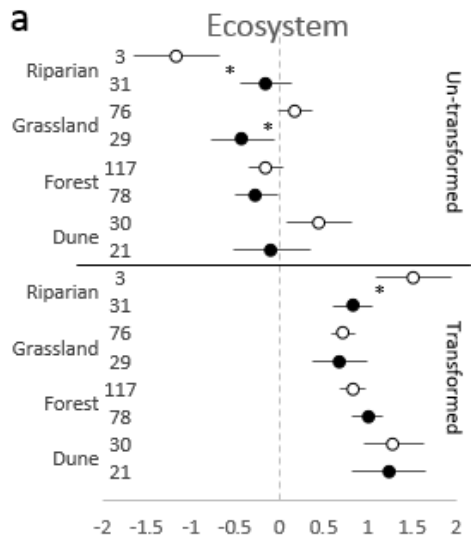


Figure 3. Subgroup analyses for ecosystem type, life form, and response metric type for both Hedges' d and transformed Hedges' d . Unfilled circles are observational studies and filled circles are removal studies with 95% confidence intervals. Stars designate significant differences and replications are listed left of data.

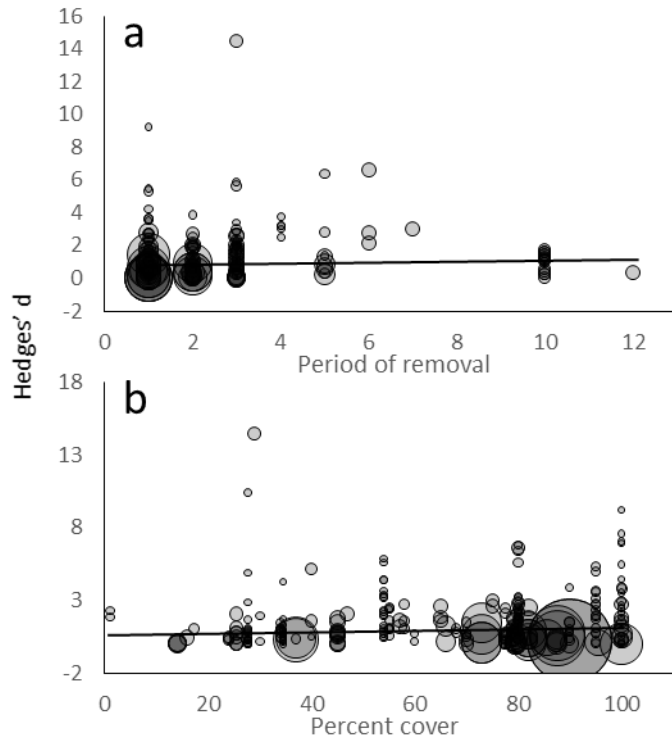


Figure 4. Meta-regressions of removal period (a) and invader percent cover (b) with Hedges' d . Circle size represents study weight in regression.

Chapter 2: System-level changes following invasion caused by disruption of functional relationships among plant and soil properties

Abstract:

The ecological impacts of invasive plants have served to justify the cost of their management, which is estimated to exceed \$1 billion annually in the US alone. However, our understanding of the ecological impacts of most invasive plants is extremely limited, and when known, interpretation is confounded with varied measurements and methods. While this can provide important information about specific components of ecosystem function, it limits our understanding of the broader scope of impacts any one species may have. Using Japanese stiltgrass (*Microstegium vimineum*) as a study system, our objectives were to 1) survey a broad suite of 29 important ecological impact metrics (EIMs), 2) identify invader cover-EIM relationships, and 3) test if the comparative reference (uninvaded or invader removal) influences interpretation. Japanese stiltgrass had the strongest effect on the plant community, followed by soil properties, soil available nutrients, and other abiotic/biotic factors. Many EIM values differed among reference types, and plant community EIMs were reduced with increasing Japanese stiltgrass cover. For example, plant biodiversity was lower in the invasion when compared to both removed and uninvaded sites; however, soil organic matter was higher only in the uninvaded site when compared to the invasion. The integrative ecosystem metric $E(c)$ also showed that the system overall was impacted by the Japanese stiltgrass invasion, and this varied among sites. Interestingly, relationships among EIMs were also changed by the presence of Japanese stiltgrass. For example, a strong correlation between pH and soil organic matter disappeared when Japanese stiltgrass was present. Together

this suggests that this invaded ecosystem functions in a different way through both individual and correlated alterations to ecosystem properties.

Introduction:

Invasive species can have strong negative impacts on ecosystem function, and are globally ranked as one of the top five threats to biodiversity (Millenium Ecosystem Assessment 2005). Specifically, impacts of invasive plants include alterations to soil nutrient dynamics, plant and animal community assemblages, and trophic dynamics (Pyšek et al. 2012). These ecological impacts, both known and perceived, serve as the motivation for the research and management of invasive plants (Mack and D'Antonio 1998). Therefore, it stands to reason that the ecological impact of invasive plants is well documented, especially considering that substantial human and economic capital are spent managing exotic plants, estimated at >\$1 billion annually in the US alone (Pimentel et al. 2005). Unfortunately, despite the volume of studies on the ecological impacts of invasive plants, our understanding of these impacts remains surprisingly limited (Hulme et al. 2013).

Of the >14,000 exotic invasive plants estimated to inhabit North America, Europe, Australia, and New Zealand, less than 200 have any quantitative assessments of ecological impact (Hulme et al. 2013). Of these studied species, ~40% include only one ecological impact metric (EIM) (e.g., native plant richness, pH), ~35% have only two EIMs, and >95% have had fewer than 5 EIMs examined (Hulme et al. 2013).

Additionally, “the rationale for which response variables [EIMs] have been included in a study does not appear clear and there are no obvious suites of variables that consistently co-occur” (Hulme et al. 2013). Our understanding of the ecological impacts for the vast

majority of invasive plant species is limited to a few inconsistent ecological parameters, leaving their broader impact largely unknown (Barney et al. 2013). This shortcoming in our understanding of the broader ecological impacts of invasive plants could have repercussions on management decisions, budget allocations, and regulations intended to limit invader impact (Quinn et al. 2013).

More than once it has been shown that the choice of EIMs can have important implications on our perception of the impact of invasion (Lockwood et al. 2001). For example, while examining the ecological impacts of saltcedar (*Tamarix spp.*), Shafroth and Briggs (2008) concluded saltcedar presence was associated with increased bird density and diversity, but decreased native plant biodiversity. Similarly, Farnsworth and Ellis (2001) concluded that purple loosestrife (*Lythrum salicaria*) presence had no effect on plant richness but a significant negative effect on overall native plant biomass. In both cases, if only one EIM was investigated, it would have greatly misrepresented the broader impact, and perhaps changed the perceived outcome as positive or negative. Single EIM studies may be warranted when a specific hypothesis about a particular species is of interest (Hulme et al. 2014), but often a broader understanding of the overall ecological impact is desired (Barney et al. 2013).

Here we study the effects of Japanese stiltgrass (*Microstegium vimineum*) on the highland forests of western Virginia. This C₄ shade tolerant annual grass is considered native to most of East Asia and is reported to be one of the top three most prevalent invasive plants in the Eastern United States (Kuppinger 2000). Japanese stiltgrass has spread across much of the forests of the Eastern United States, and now occupies an estimated 260,000 hectares east of the Mississippi River (Kuppinger 2000). With nine

EIMs independently studied (Kourtev et al. 1999, 2003, Baiser et al. 2008, Marshall et al. 2009, Marshall and Buckley 2009, Strickland et al. 2010, Brewer 2010, Simao et al. 2010), only cheatgrass (*Bromus tectorum*) and common reed (*Phragmites australis*) are better studied (Hulme et al. 2013). Therefore, one may assume we have a strong understanding of the ecological impact of Japanese stiltgrass.

Unfortunately, the 9 EIMs were studied in separate populations precluding our ability to understand the potential network of interactions and feedbacks among EIMs following invasion (Le Maitre et al. 2011). As Hulme et al. (2013) stated, “response variables should not be assessed in isolation but viewed as inter-related factors of ecosystems that might be directly or indirectly impacted by an alien plant.”

Of additional concern is that some studies have found that impact to EIMs varies with invader abundance (typically measured as percent ground cover) (Thiele et al. 2011), but only 18% of invasive plant impact studies consider invader cover (Tekiel and Barney unpublished data). Of those studies, >50% found that impact magnitude varied with invader cover. Of existing studies examining EIMs of Japanese stiltgrass, cover level was never a factor in measuring impact. Thus, the influence of invader cover level for all invasive plants should be further examined because understanding abundance-impact relationships may uncover important thresholds that could be exploited during management or restoration (Barney et al. 2013).

Identifying ecological impacts attributable to invasive plants requires comparing the invaded patch to an alternative patch (often referred to as native or pre-invaded) without the invader (Barney et al. 2015; Kumschick et al. 2015). The two most commonly used references to benchmark invader impacts are 1) a nearby uninvaded site

that is assumed to represent the pre-invaded system, or 2) a portion of the invaded patch removed. In the case of Japanese stiltgrass, 66% of studies used observational uninvaded references and 33% used removal references; thus, different methods were used to determine Japanese stiltgrass impacts. Each method has benefits and drawbacks regarding their underlying assumptions (Kumschick et al. 2015), which may affect their utility and interpretation, as well as the magnitude and direction of the resulting impacts.

To obtain a more holistic understanding of the impacts of invasive plants our objectives were to 1) use a multi-metric approach to gain a broader understanding of the consequences of invaders, including EIMs that do not change, 2), determine if EIMs vary with invader cover, and 3) determine if the reference type changes interpretation, all of which have been identified as important, yet understudied components of invader impact (Kumschick et al. 2015).

Methods:

Site selection:

We identified Japanese stiltgrass patches within representative habitats of the Ridge and Valley section of Virginia mountain forest ecosystem that met the following criteria: 1) an invasion patch large enough to contain the study design (see below) with all plots >1m from the invasion front; 2) no clear dispersal boundaries among plots within the invasion (e.g., large felled tree, waterway); 3) Japanese stiltgrass being dominant in the area; and 4) an adjacent uninvaded reference site that is most likely uninvaded due to lack of propagule pressure, not due to inherent abiotic differences (White and Walker 1997, Powell et al. 2013).

The most southern patch was in Pandapas Pond Recreation Area (PAN) located in Montgomery County, Virginia (37.281088N, 80.475236W). The forest canopy of this location is primarily red maple (*Acer rubrum*), white oak (*Quercus alba*), and red oak (*Quercus rubra*). The understory community composition is typical of Appalachian forests of Virginia with total richness of 78 species (Brown and Peet 2003). Soils are a Berks-Weikert composition on slopes of 15 to 25 percent (Web Soil Survey). The reference site was selected beyond a 0.5 m wide ephemeral creek likely acting as a barrier to dispersal, but was otherwise similar to the invaded sites in canopy composition, soils, and aspect.

The second patch was in Peaks of Otter Recreation Area (POT) in Jefferson National Forest, Bedford County, Virginia (37.442586N, -79.612103W). The forest canopy composition is primarily white oak, red oak, and mockernut hickory (*Carya tomentosa*). This location also has a forest understory community typical of the area and similar to PAN. The soils of this location are shallower and rockier than the two other locations, with less than 2 cm of topsoil in some areas. The reference was located adjacent (> 5m) to the Japanese stiltgrass invasion.

The most northern patch, Babbling Springs (BBS) Recreation Area (37.926001N, -79.605503W), was located in George Washington National Forest, Rockbridge County, Virginia. The canopy composition of this location is primarily white oak, red oak, and white ash (*Fraxinus americana*). Again, the community of this understory is typical of Virginia Appalachian forests. Soils, similar to PAN, were a Weikert-Berks-Rough complex on moderate slopes (Web Soil Survey). The reference site was defined along the same ephemeral stream where Japanese stiltgrass had not yet invaded. All uninvaded

sites were similar in native understory plant composition, soil type, and overstory composition.

Site Layout:

At each location we established two treatments within the invasion patch, and two within the uninvaded site. Ten replicates of each treatment were placed in PAN and six were placed in both POT and BBS. Within the invasion patch we installed 3.50 x 1.75m split plots that were randomly assigned to 1.75 x 1.75m invaded (IN) or invader removal treatments (RE). IN plots were left un-manipulated, while RE plots were managed to remove Japanese stiltgrass by hand removing, which imposed minimal disturbance while achieving ~0% cover of Japanese stiltgrass. Removal occurred in mid-May and mid-June while plants were <3 leaf stage. Thus, the Japanese stiltgrass was absent in RE plots for <1yr, which is a common duration in most existing studies (34% of removal studies; Tekiela and Barney, unpublished data). Two independently randomized 1.75 x 1.75m treatment types were installed in the uninvaded sites. Uninvaded (UN) reference plots were left untouched to act as a reference to the invaded patch. Seeded plots (SE) were sown with Japanese stiltgrass seed at a similar density to the current invasion to ensure the uninvaded reference sites were vulnerable to invasion and were only uninvaded due to a lack of propagules, not due to abiotic differences (Eschtruth and Battles 2011). To determine what density to seed SE plots, average density of Japanese stiltgrass was assessed in the invasion patch to be 1264 plants m⁻². Therefore, SE plots were seeded in May 2013 prior to germination by evenly dispersing 3871 seed across the 3.06m² plot using a granule shaker. Thus, seeded plots were only used to assess the quality of the UN reference site and were not considered further during analyses. The outer 0.375m (2.06

m²) edge of each plot was designated as a border to reduce edge effects, with only the inner 1m² used for data collection. This dimension was chosen based the average height of Japanese stiltgrass in these habitats.

Selection & Method of Metric Collection:

Data collection occurred in late July 2013 during the peak growing season to capture the greatest influence of Japanese stiltgrass. We selected the most commonly measured EIMs that could be practically implemented on a large scale within a brief sampling period, while still representing the primary suite of EIMs present in the literature (Sup. Table. 1). All recorded EIMs represent environmental characteristics implicated in ecosystem function, and were grouped into the general categories of plant community, soil physical property, plant available soil nutrients, or other abiotic/biotic parameters. Using data from multiple impact studies and simulated data, Barney et al. (2013) used a bootstrap method to measure variation in estimated total ecosystem impact, and suggested measuring >15 EIMs to best capture broader invasive plant impacts to the invaded ecosystem regardless of EIM identity. Therefore, by measuring 29 EIMs, we exceeded this minimum and should be capable of making robust conclusions on the ecological impact of Japanese stiltgrass regardless of the selection of EIMs.

Methodology for collecting and analyzing each EIM was taken from the literature and adapted as needed to our study (Sup. Table 1). All methodology used was standard except for earthworm extraction and infiltration rate which were combined to reduce disturbance to the plot. In place of water for the infiltration test, mustard powder solution was used to estimate infiltration rate, while simultaneously sampling earthworms.

Data Analysis:

Similar to the majority of invasive plant impact studies, we performed univariate techniques to test for treatment effects of each EIM independently. For each EIM, we used ANOVA with treatment (UN, RE, IN) and location (PAN, POT, BBS) as fixed effects (Bolker et al. 2009) with post-hoc Tukey's honestly significant difference ($\alpha=0.05$) for mean separation. To test if Japanese stiltgrass cover influenced individual EIMs, ANCOVA was performed on IN plots using Japanese stiltgrass cover, the quadratic term of Japanese stiltgrass cover (to test for non-linearity), and location as independent variables for each dependent EIM.

To estimate system-level impact we derived the integrated impact value, $E(c)$, according to Barney et al. (2013). $E(c)$ is designed to integrate any number of EIMs as a function of invasive plant cover. A modified version of $E(c)$ was calculated using UN or RE plots independently as references (\bar{n}) to test if different $E(c)$ result from different reference types—in other words how ecologically different are the uninvaded and removal plots. A Δk was calculated for each EIM as the difference between IN and a reference (UN or RE); which was then integrated into $E(c)$ by determining the geometric mean of Δk s (i.e., $E(c) = \text{geometric mean of } \Delta k\text{s}$). Therefore, $E(c)$ can only be calculated for each IN plot. ANCOVA was then performed separately using UN and RE reference plots on $E(c)$ using Japanese stiltgrass cover and location as independent variables.

We also used a principle component analysis (PCA) to interpret system-level responses (at least within the context of the 29 EIMs we measured) to Japanese stiltgrass invasion and reduce the number of ecological components. PCA was performed using Japanese stiltgrass percent cover and all EIMs listed in Table 1 and varimax rotated to maximize sums of squared variance for each loading value (Sup. Figure 1). The

component reduction was performed using a combination of a scree plot, variance explained, and comprehensibility of eigenvector loadings to determine how many components parsimoniously represented the data (Cattell 1966). Five components accounted for 68% of the variation and generally grouped EIMs into the following: component 1 = carbon pools and cycling; component 2 = overall plant diversity and richness; component 3 = pH and cations; component 4 = invasive plant richness and cover; and component 5 = native plant richness and cover (Sup. Table 2). Additionally, within each component a “flagship” EIM was chosen using the highest loading value. In cases where multiple EIMs loaded strongly and relatively equally to a single component, we chose the EIM based on ecological principles to select a flagship EIM that best represented the grouping (e.g., pH instead of Ca). Flagship EIMs were as follows: 1 = organic matter; 2 = diversity index; 3 = pH; 4 = invasive richness; and 5 = native cover.

To our knowledge, no study has attempted to determine if an invasive plant modifies interactions among EIMs; the so-called interconnectedness (Hulme et al. 2013). Therefore, we performed a quadratic discriminant analysis, which allows the variation structure among treatments to be independent; allowing the correlation structure within treatment to be driven by changes due to Japanese stiltgrass (Williams 1983). It also allows us to examine the similarities or difference between treatments and if the five components and flagship EIMs are successful at predicting Japanese stiltgrass presence/absence when compared to using the full suite of EIMs. To better understand the correlation structure of EIMs within each treatment and the predictive quality of EIM's, a quadratic discriminant analysis was performed separately on flagship EIMs, components, and all EIMs using treatment (IN, UN, RE) as the discriminating factor to

test how different the correlation structure of treatments were, and if our EIM reduction had similar invasion predictive qualities. Within treatment correlation structure was recorded for each of the three discriminant analyses. To determine if within treatment correlation structure is statistically different, an equality of covariance test was performed on the correlation structure for each group (i.e., flagship EIMs, components, all EIMs). All statistical analyses were run in JMP v.11 (SAS Institute Inc.) except for the equality of covariance test, which was performed in R v.3.1.2 (R Development Core Team).

Results:

By August, Japanese stiltgrass within seeded plots established and began flowering, suggesting uninvaded reference sites were susceptible to invasion by Japanese stiltgrass. During the peak growing season in July, Japanese stiltgrass cover in IN plots averaged $72 \pm 21\%$ (standard deviation), ranged from 18 to 95% and was consistent across sites ($p > 0.05$). Removals reduced Japanese stiltgrass cover in RE plots to $1 \pm 0\%$ with a range of 0 to 1% in POT and BBS, and $4 \pm 3\%$ with a range 1 to 10% in PAN. Additionally, at all sites UN plots did not have any encroachment of Japanese stiltgrass during the study and remained uninvaded.

The plant community EIMs, plant diversity index (H'), plant diversity index without Japanese stiltgrass (H_r'), native plant richness, invasive plant richness, and bare ground were all reduced by the presence of Japanese stiltgrass when compared to UN plots (Figure 1; Table 2). Plant diversity index (H'), total plant richness, native plant richness, bare ground and native plant cover were reduced by the presence of Japanese stiltgrass when compared to RE plots (Figure 1; Table 2). Across all sites, 34 plant species were identified in RE plots that were not present in IN plots (Sup Table 3). Other

abiotic/biotic EIMs were also impacted by the invasion. For example, light penetration and litter abundance were reduced by the presence of Japanese stiltgrass when compared to UN plots (Figure 1; Table 2), while only light penetration was reduced by Japanese stiltgrass when compared to RE plots (Figure 1; Table 2). Of the soil property EIMs, pH was higher and organic matter was lower in the Japanese stiltgrass invasion, when compared to UN plots (Figure 1; Table 2). Soil potassium, calcium, magnesium, manganese, copper, and boron were affected by Japanese stiltgrass presence when compared to UN plots (Table 2), but only potassium and manganese were different between IN and RE (Table 2).

Additionally, we compared all EIMs between RE and UN plots to determine the similarities or differences between the two most commonly used reference types. Three of eight plant community EIMs (percent bare ground, percent native cover, percent other invasive cover) were different. The species present in UN but not RE plots were predominately trees, while species present in RE but not UN plots were predominantly fast growing ruderal species. One of six other abiotic/biotic EIMs (litter abundance) and one of six soil property EIMs (pH), were different between RE and UN (Figure 1; Table 2).

Plant diversity index (H'), bare ground, and native plant cover decreased linearly with increasing Japanese stiltgrass cover, while total plant richness and native plant richness had a negative quadratic relationship with increasing invasive cover that peaked at 48% and was lowest at maximum Japanese stiltgrass cover (Figure 2); no other EIMs varied with Japanese stiltgrass cover.

The total ecosystem impact $E(c)$ (the integration of multiple EIMs into a single value *sensu* Barney et al. (2013)) values varied between the reference types ($p=0.0053$); therefore, results are reported separately for UN and RE references (Sup. Table 4). $E(c)$ with UN as the reference was 1.61 ± 0.06 for PAN, 1.85 ± 0.11 for POT, and 1.68 ± 0.06 for BBS. Using RE as reference, $E(c)$ was 1.42 ± 0.04 for PAN, 1.81 ± 0.05 for POT, and 1.51 ± 0.05 for BBS. The similarity in PAN and BBS $E(c)$ values were associated with the overlapping clustering of points in the PCA while the dissimilar POT did not overlap in the PCA (Sup. Figure 1). Using UN as reference, $E(c)$ did not vary with Japanese stiltgrass cover ($p = 0.0679$), though $E(c)$ did vary with Japanese stiltgrass cover with RE as a reference ($p = 0.0295$; Sup. Figure 2).

Quadratic discriminant analysis with flagship EIM's, components, and all EIM's resulted in successful discrimination of treatment (IN, UN, RE) with Wilks' Lambda test scores of $p<0.0001$. However, the proportion of misclassified plots after training varied: 13.9% of plots were misclassified using flagship EIM's; 17.5% of plots were misclassified using components; and 0% of plots were misclassified using all EIMs (Figure 3). With flagship variables, RE plots were most commonly misclassified as UN plots (7.7%) followed by UN plots misclassified as RE plots (3.0%). Components also had RE plots most commonly misclassified as UN (8.8%) but IN plots misclassified as RE plots was the second most common misclassification (5.3%). The within treatment correlation structure was significantly different for all discriminant analyses with all EIMs ($p<0.0001$), flagship EIMs ($p<0.0001$), and components ($p<0.0001$; Figure 4) suggesting EIMs did not vary similarly within different treatments (Sup. Table 5).

Discussion:

The invasion of Japanese stiltgrass has clear ecological impacts on forests of the Eastern US. Examined individually, plant EIMs were most strongly impacted by Japanese stiltgrass, but all impact categories had at least one EIM altered by the invasion. Multivariate analysis indicated a clear distinction between the invaded site and either reference type, suggesting an ecosystem-level change following invasion that was not immediately alleviated by removing the invader. Additionally, several plant metrics were negatively correlated with increasing Japanese stiltgrass cover, while all other metrics were either unchanged, or changed in a binary (presence/absence of Japanese stiltgrass) fashion – suggesting that ecosystem processes can respond in either a density-dependent or independent fashion, at least given the range of covers observed here. Importantly, our analysis also revealed that removal and uninvaded references are not equitable, which has clear methodological implications for estimating invasive plant impacts.

Proportionally, the plant community exhibited the largest magnitude change following Japanese stiltgrass invasion, suggesting resident plants are most susceptible to forest invaders. The amount of bare ground, native diversity, native cover, and total richness were all reduced in the invaded system, similar to other Japanese stiltgrass invasion studies (Adams and Engelhardt 2009, DeMeester and Richter 2009, Flory 2010). Litter abundance decreased and soil microbial activity and earthworm abundance were unaffected by the invasion, similar to some previous results (Kourtev et al. 1999); however, these similarities existed at only one of two sites studied, suggesting the response of these metrics is site dependent and may not always be driven primarily by Japanese stiltgrass. As has been shown in multiple studies, pH and Mg (Ehrenfeld et al.

2001, McGrath and Binkley 2009) increased following Japanese stiltgrass invasion, while TOC, CEC, P, K, Ca, and soil moisture all responded differently in our study compared to others (McGrath and Binkley 2009).

Looking beyond single impact metrics, our multivariate analysis reduced 29 EIMs into five ecologically relevant components: 1 = carbon pools and cycling; 2 = plant community diversity and richness; 3 = soil pH and cations; 4 = invasive plant richness and cover; and 5 = native plant richness and cover. Additionally, all discriminant analyses differentiated the three treatments (IN, UN, RE), suggesting treatments displayed unique characteristics across all EIMs. Importantly, this analysis revealed that Japanese stiltgrass invasion altered the broader function of the forest across a range of plant, soil, and other environmental characteristics. Though using the complete suite of EIMs was most successful and led to no errors in discrimination of treatments, use of components or flagship variables was nearly as successful. This suggests an even more limited suite of metrics than was previously suggested (Barney et al. 2013) may effectively represent broad ecological change to an invaded ecosystem. It remains unknown whether these same variables would be ideal indicators of environmental change for other species invading different systems. Although our findings raise the possibility that a small suite of carefully chosen indicator variables could serve to assess broad-scale environmental changes in invaded systems, this should be tested in more species.

The integrated metric $E(c)$ is designed to estimate the total magnitude of impact to an invaded ecosystem (Barney et al. 2013). Integrating the single parameters into $E(c)$ in our study, the first to do so, showed that the locations PAN and BBS had similar $E(c)$

values, both of which were different than POT. Interestingly, the distribution of points showed similar patterns in PCA space: PAN and BBS overlapped, while POT was separate. This suggests that $E(c)$ may allow discrimination of ecosystem-level impact among populations. However, more locations are needed to test this hypothesis, which lends itself to globally distributed experiments (e.g., Barney et al. 2015).

Ecosystems are defined not only by the organisms that inhabit an area and the resources available; but also by the interactions of organisms and resources (O'Neill et al. 1986). All ecosystems have a structure that is defined by the pools and fluxes of energy and matter in the system (Hannon 1973). The addition or removal of any one species or resource from a system can impact the strength of other interactions, especially when that species is a large component of net primary productivity (Wardle and Bonner 1999). Therefore, to better understand the impact of invasive species on an ecosystem, the interactions among EIMs are just as important as their individual changes in magnitude and direction. Not only did we find that invasion changed EIMs using univariate and multivariate analysis, but we also found that correlations among environmental variables changed in the presence of Japanese stiltgrass. For example, within our flagship EIMs, organic matter and pH were poorly correlated in the uninvaded site, suggesting there was little or no relationship between these two EIMs and they varied independently. However, in the presence of Japanese stiltgrass, organic matter and pH became strongly positively correlated (i.e., plots with increased pH also increased in organic matter) even though organic matter decreased on average in the invasion. Japanese stiltgrass increases soil pH when invading, as found in this study and by others (Kourtev et al. 1999, Ehrenfeld et al. 2001), which is well known to change microbial community composition and litter

decompositions rates (Högberg et al. 2007). Because pH varied less and was more acidic in the uninvaded landscape, the relationship between pH and soil organic matter may have been weaker. However, following the invasion of Japanese stiltgrass and the subsequent less acidic soil pH, soil organic matter was lower; and became coupled. A strong negative correlation between native plant cover and plant diversity (H') in uninvaded sites shifted to a strong positive correlation in invaded sites. When Japanese stiltgrass was present, increased native plant cover indicated decreased exotic plant cover and therefore increased native plant diversity. However, in the absence of invaders, increased native plant cover generally corresponded with dominance of a few species and reductions in diversity. While we cannot identify the mechanism underlying these relationships and are not able to confirm causation, our data suggest that invaded ecosystems function in a different way than uninvaded systems. Further research should look to understand what role invaders play in the correlation structure of important ecological functions in other systems.

A limited number of studies have demonstrated that some ecosystem processes vary with invader density or cover (Greene and Blossey 2011, Hart and Holmes 2013). The vast majority of existing impact studies do not report invader cover, but the common dichotomous 'invaded'/'uninvaded' comparison may disregard important ecological relationships. For example, Thiele et al. (2011) showed that species richness had a negative non-linear relationship with *Rosa rugosa* cover. In our study system, plant diversity (H'), bare ground, and native plant cover all decreased with increasing Japanese stiltgrass cover and total plant richness and native plant richness had a quadratic relationship with Japanese stiltgrass cover. No other EIM's in any other category

responded to increasing cover of Japanese stiltgrass. The plant community-invader cover relationship may be due to the direct competition that occurs between increasing cover of the invader with the resident plant community. Other ecosystem processes (e.g., litter decomposition) are either less labile or are indirectly impacted by Japanese stiltgrass.

The EIM responses to Japanese stiltgrass fall into two categories: 1) density dependent, where the magnitude changes to increasing invader cover (e.g., native richness); 2) and density-independent (e.g., pH), where the magnitude only changes in the presence of Japanese stiltgrass, regardless of abundance, at least over the surveyed range of covers. Currently, of the 11 studies that explicitly examined the influence of cover level of an invasive species, 91% examined only plant metrics and 90% of those showed a negative relationship of EIMs with increasing cover. Only purple loosestrife was shown to not impact plant richness (Farnsworth and Ellis 2001). The one study examining a non-plant EIM studied frog mass with increasing cover of an invasive plant and saw a negative relationship (Maerz et al. 2005). Unfortunately, no other studies examine non-plant metrics in this way so little can be said about the overall influence on non-plant metrics beyond the results of this study. It should be noted that very few plots had <50% Japanese stiltgrass cover, which could have led to the binary response seen from some metrics (i.e., they did not vary with cover); However, typical Japanese stiltgrass invasions occur at the high levels of cover seen in this study, so our results are representative, but may lack power to test for all cover-impact relationships. Future studies should seek to evaluate broader cover-impact relationships (Barney et al. 2013, Kumschick et al. 2015). Importantly, the potential for density dependent and independent changes to Japanese stiltgrass invasion has important ecological and management implications. First, some

metrics change immediately following invasion, while others accrue with increasing invader cover (Levine et al. 2006). Secondly, this can be used to justify management of small low-density incipient populations to mitigate the density-dependent changes before they accumulate larger magnitude changes.

To estimate the impact of an invasive plant, the invaded patch is compared to some benchmark, which most often is an adjacent uninvaded patch or removing the invader (Hulme et al. 2013). Though it is rarely tested, the reference site identity may result in different conclusions on invader impact due to their assumptions and limitations (Kumschick et al. 2015) as has been shown between removal and addition reference sites (Truscott et al. 2008). Although removal and uninvaded references are commonly used to estimate invasive plant impact, our results suggest these two benchmarks are not ecologically equivalent, at least in our timeframe of <1 year. In our study, the discriminant analysis differentiated 84% of UN and RE plots using flagship EIMs and components, and 100% using all EIMs, suggesting a clear distinction between an uninvaded site and one with the invader recently removed. Additionally, $E(c)$ values varied whether removal or uninvaded plots were used as the reference further suggesting the reference types are not equitable. If these reference plots were equitable, the discriminant analysis would not differentiate between them. Removing Japanese stiltgrass did not bring the plot back to the uninvaded state, suggesting that while some components of the ecosystem change immediately following invasion, they do not return following removal of the invader. However, a longer term study is necessary to fully identify these relationships.

The act of removing a plant species regardless of how carefully performed is itself a disturbance to the system. While reviewing competition studies, Aarssen and Epp (1990) looked at removal methodologies to elucidate competitive relationships, and were concerned that the disturbances associated with removal studies affected results and conclusions. In fact, differing removal methodology may influence the response of the surrounding community (Skurski et al. 2013). Even if the plant can be removed with no physical disturbance, the opening of the canopy itself could have lasting effects. This may partly explain the difference seen in plant EIMs between reference types. Other invasive cover was greater in RE (5.1%) than IN (2.2%) and UN (1.3%), suggesting that other invasive cover increased following removal. We saw a similar pattern in native cover in RE plots, which was nearly twice (31.5%) that of IN and UN (17.9, 18.5% respectively). Interestingly, the species that increased following removal were generally ruderal fast growing species. In contrast, trees were more common in the UN plots than either IN or RE, suggesting more long-term effects on slow-growing k-selected species, which others have shown following Japanese stiltgrass invasion (Beasley and McCarthy 2011). Skurski et al. (2013) found that the inherent disturbance associated with removal of a grassland herbaceous invasive species only encouraged further invasion of exotic grasses. Therefore, the unavoidable disturbance of removing Japanese stiltgrass may influence the results and their interpretation, and thus the magnitude and interpretation of invader impact if using removal as the benchmark.

Even if disturbance is minimized, removing an invasive plant to represent an uninvaded community may not fully remove the influence of the invader (Corbin and D'Antonio 2012). In our system, removals were performed five months before data was

collected, which is a common timeframe in ecological impact studies (Tekiel and Barney, unpublished data). However, this presents an obvious temporal limitation and assumes near instant return rates (i.e., invaded changes revert to uninvaded state instantly following invader removal), which may not occur. Instead, legacy effects, which are the persistent effects of a species even after its removal, disrupt the ability and rate of return of a system to the uninvaded state (Cuddington 2011). Elgersma et al. (2011) showed that the historical presence of Japanese barberry (*Berberis thunbergii*) had lasting effects on the soil microbial community structure even two years after its extirpation from the system. In contrast, we saw no impact to microbial biomass or activity by Japanese stiltgrass in our system. The differences in pH and leaf litter abundance we observed between UN and RE may be explained by legacy effects. For both EIMs, RE values remained similar to IN but distinct from UN. Similar legacies have been reported in leaf litter abundance in common reed (*Phragmites australis*) (Holdredge and Bertness 2010), and similar to our results, Japanese stiltgrass has been previously shown to reduce overall leaf litter depth in forest understory systems (Kourtev et al. 1998). Japanese stiltgrass is also well known to increase pH in soils (Kourtev et al. 1999, Ehrenfeld et al. 2001) as was recorded at our sites (IN = 5.78, RE = 5.44, UN = 4.91). Additionally, McGrath and Binkley (2009) suggest changes in pH are only distinguishable after 6 months of Japanese stiltgrass presence which may explain the lack of difference between invaded and removed treatments. Alternatively, though plant metrics were most greatly impacted by Japanese stiltgrass, they may also have been able to respond within five months of removals and return to a similar state to uninvaded plots in a short period of time due to their lability. Primarily slow to establish tree species were found in UN plots and not RE

plots. DeMeester and Richter (2009) also saw a quick response of native plant cover and bare ground cover after removing Japanese stiltgrass from the plot, further suggesting plant community composition has limited legacy effects when compared to soil and ecosystem EIMs.

Our study suggests that Japanese stiltgrass changes the invaded ecosystem, and that ecosystem pools and fluxes respond in both density dependent and independent patterns. Important for future invasive plant impact studies is to consider the following: 1) a broad suite of relevant ecosystem parameters; 2) identify parameter responses to invader abundance; and 3) thoughtfully consider the reference benchmark. Enhanced understanding of the ecological impacts of invasive plants allows land managers to more optimally prioritize the utilization of limited resources to focus on highly impactful invasive species, which will therefore lead to more efficacious and cost-effective management.

Acknowledgements:

We would like to thank Carissa Ervine, Elise Benhase, Eugene Dollete, Larissa Smith, Morgan Franke, Rose Peterson, and Ryan Schmitt for help in the field. We thank the National Forest Service for their assistance in obtaining sites. We also thank Dan Atwater for helpful comments on the manuscript. We also acknowledge the Global Invader Impact Network (GIIN) managed by our research group, of which this study is a component.

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Table 1. Statistical results for independent EIM ANOVA tests based on treatment and location.

Measured EIMs	Treatment			Location	
	DF	F-value	p-value	F-value	p-value
Plant community					
Diversity index (H')	2	82.23	0.0001	1.82	0.1712
Diversity index w/o invader	2	4.93	0.0103	2.43	0.0964
Richness	2	7.85	0.0009	0.55	0.5795
Native richness	2	9.00	0.0004	0.98	0.3805
Invasive richness	2	12.73	0.0001	24.38	0.0001
Bare ground (%)	2	153.30	0.0001	2.79	0.0695
Other invader cover (%)	2	4.03	0.0227	7.75	0.0010
Native cover (%)	2	6.03	0.0041	0.96	0.3898
Other biotic and abiotic parameters					
Microbial biomass (mg kg ⁻¹)	2	0.16	0.8525	49.75	0.0001
Microbial activity ($\mu\text{g CO}_2$ 10g soil ⁻¹ hr ⁻¹)	2	1.16	0.3195	36.85	0.0001
Light penetration (%)	2	16.23	0.0001	3.36	0.0411
Litter abundance (g m ⁻²)	2	4.47	0.0154	3.95	0.0243
Litter decomposition (%)	2	0.18	0.8386	17.39	0.0001
Earthworms m ⁻²	2	2.47	0.0936	2.45	0.0952
Soil Physical Properties					
Infiltration rate (cm min ⁻¹)	2	1.94	0.1530	12.3	0.0001
Soil moisture (%)	2	1.36	0.2636	13.03	0.0001
pH	2	8.18	0.0007	14.15	0.0001
Organic matter (%)	2	3.45	0.0381	376.63	0.0001
TOC (ppm)	2	0.15	0.9003	100.64	0.0001
CEC (meq 100g ⁻¹)	2	0.23	0.7982	3.06	0.0542
Plant Available Soil Nutrients					
N (%)	2	0.17	0.8415	166.62	0.0001
P (ppm)	2	2.40	0.0988	19.35	0.0001
K (ppm)	2	8.99	0.0004	37.37	0.0001
Ca (ppm)	2	6.63	0.0025	12.27	0.0001
Mg (ppm)	2	5.13	0.0087	15.60	0.0001
Zn (ppm)	2	1.26	0.2901	0.60	0.5503
Mn (ppm)	2	7.38	0.0013	3.50	0.0362
Cu (ppm)	2	4.43	0.0160	89.23	0.0001
Fe (ppm)	2	0.15	0.8583	3.12	0.0512
B (ppm)	2	4.38	0.0167	13.92	0.0001

Table 2. EIM means and standard errors in uninvaded, removed, and invaded plots. Means separated using post-hoc Tukey's honestly significant difference. Means with different letters are statistically different.

Measured EIMs	Uninvaded		Removed		Invaded	
	Mean	SE	Mean	SE	Mean	SE
Plant community						
Diversity index (H')	2.240	0.078a	2.099	0.101a	0.831	0.079b
Diversity index w/o invader	2.224	0.077a	2.027	0.103ab	1.776	0.125b
Richness	12.36	0.636ab	14.50	0.664a	10.63	0.752b
Native richness	11.09	0.569a	11.68	0.643a	8.091	0.705b
Invasive richness	1.273	0.248b	2.818	0.387a	2.545	0.261a
Bare ground (%)	79.9	2.4a	61.1	4.0b	7.5	2.7c
Other invader cover (%)	1.3	0.3a	5.1	1.7b	2.2	0.8ab
Native cover (%)	18.5	2.5b	31.5	3.6a	17.9	3.2b
Other biotic and abiotic parameters						
Microbial biomass (mg kg ⁻¹)	0.249	0.034a	0.242	0.028a	0.233	0.022a
Microbial activity ($\mu\text{g CO}_2$ 10g soil ⁻¹ hr ⁻¹)	0.0668	0.0054a	0.0684	0.0070a	0.0593	0.0070a
Light penetration (%)	86.90	6.47a	67.63	6.29a	41.15	4.847b
Litter abundance (g m ⁻²)	24.42	1.050a	21.04	0.9855b	20.78	0.9725b
Litter decomposition (%)	14.71	1.33a	15.42	2.46a	16.14	1.55a
Earthworms m ⁻²	10.2	4.65a	6.85	2.60a	0.7616	0.7616a
Soil Physical Properties						
Infiltration rate (cm min ⁻¹)	3.748	1.132a	1.789	0.8684a	1.962	0.6718a
Soil moisture (%)	30.56	2.066a	34.73	2.016a	32.89	2.228a
pH	4.908	0.0638a	5.435	0.1329a	5.782	0.2807b
Organic matter (%)	5.559	0.7098a	4.968	0.6475b	4.781	0.5798b
TOC (ppm)	2.999	0.3665a	3.129	0.3987a	3.089	0.4820a
CEC (meq 100g ⁻¹)	5.738	0.6134a	6.049	0.5101a	6.245	0.5355a
Plant Available Soil Nutrients						
N (%)	0.2167	0.0288a	0.2221	0.0260a	0.2131	0.0271a
P (ppm)	2.318	0.1527a	2.045	0.0455a	2.227	0.1127a
K (ppm)	36.32	3.173b	50.14	5.282a	60.18	8.080a
Ca (ppm)	161.6	10.21b	373.0	70.13ab	623.1	166.7a
Mg (ppm)	36.41	3.558b	84.45	21.17ab	165.1	56.18a
Zn (ppm)	1.364	0.062a	1.727	0.188a	6.577	4.454a
Mn (ppm)	9.191	0.880a	10.20	0.834a	14.19	1.262b
Cu (ppm)	0.414	0.039a	0.505	0.060ab	0.527	0.065b
Fe (ppm)	20.40	1.999a	24.00	7.338a	25.64	9.608a
B (ppm)	0.1591	0.0126a	0.2182	0.0333ab	0.3409	0.0839b

Supplemental Table 1. Suite of ecological impact metrics (EIMs) measured within each treatment (invaded, uninvaded, removed), their categorization, and methodological details.

Measured EIM	EIM Categorization	Additional Details	Reference
Diversity index (H')	Plant community	H' calculated on a percent area basis	(Spellerberg and Fedor 2003)
Diversity index w/o invader	Plant community	H' calculated on a percent area basis	
Richness	Plant community	USDA plants database used to determine nativity (USDA 2014)	(Flory 2010)
Native richness	Plant community	USDA plants database used to determine nativity	
Invasive richness	Plant community	USDA plants database used to determine nativity	
Bare ground (%)	Plant community		
Other invader cover (%)	Plant community		
Native cover (%)	Plant community		
Microbial biomass (mg kg ⁻¹)	Other biotic/abiotic	Chloroform fumigation	(Jenkinson and Powlson 1976)
Microbial activity (μg CO ₂ 10g soil ⁻¹ hr ⁻¹)	Other biotic/abiotic	Soil induced respiration	(Anderson and Domsch 1978)
Light penetration (%)	Other biotic/abiotic	$\frac{PAR_{above} - PAR_{below}}{PAR_{above}}$	(Machado and Reich 1999)
Litter abundance (g m ⁻²)	Other biotic/abiotic	Collected from 0.0625 m ² border	
Litter decomposition (%)	Other biotic/abiotic	0.0625 m ² area	(LIDET 1995)
Earthworms m ⁻²	Other biotic/abiotic	0.00785m ² area with 0.32L mustard solution (10g L ⁻¹)	(Eisenhauer and Scheu 2008)
Infiltration rate (cm min ⁻¹)	Soil physical property	0.1m diameter PVC ring driven 0.08m into soil	(SEMCOG 2008)
Soil moisture (%)	Soil physical property	Dynamax TH300	Dynamax Manual
pH	Soil physical property		Virginia Extension
Organic matter (%)	Soil physical property		Virginia Extension
TOC (ppm)	Soil physical property		Virginia Extension
CEC (meq 100g ⁻¹)	Soil physical property		Virginia Extension
N (%)	Soil nutrient		Virginia Extension
P (ppm)	Soil nutrient		Virginia Extension
K (ppm)	Soil nutrient		Virginia Extension
Ca (ppm)	Soil nutrient		Virginia Extension
Mg (ppm)	Soil nutrient		Virginia Extension
Zn (ppm)	Soil nutrient		Virginia Extension
Mn (ppm)	Soil nutrient		Virginia Extension
Cu (ppm)	Soil nutrient		Virginia Extension
Fe (ppm)	Soil nutrient		Virginia Extension
B (ppm)	Soil nutrient		Virginia Extension

Supplemental Table 2. Principle component analysis (PCA) selected components. Flagship EIM values are bolded for each component.

	Component 1	Component 2	Component 3	Component 4	Component 5
Species of interest cover (%)	-0.0246	-0.7462	0.2128	0.2386	-0.2206
Diversity index (H')	0.0449	0.8892	-0.2227	-0.0518	0.1010
Diversity index w/o invader	0.1207	0.7651	-0.1656	0.1877	-0.0430
Richness	-0.0751	0.5961	-0.1210	0.2534	0.6548
Native richness	-0.1090	0.6319	-0.1524	-0.1443	0.6367
Invasive richness	0.0640	-0.0063	0.0533	0.8843	0.1163
Bare ground (%)	0.1088	0.8272	-0.2513	-0.3108	-0.1318
Other invader cover (%)	-0.0888	0.0311	-0.0298	0.6650	0.0259
Native cover (%)	-0.1496	-0.0981	0.0687	-0.0933	0.7792
Microbial biomass (mg kg ⁻¹)	0.7752	0.1206	0.3387	0.0136	0.1370
Microbial activity (μg CO ₂ 10g soil ⁻¹ hr ⁻¹)	-0.6902	0.0468	-0.2035	-0.0083	-0.0986
Light penetration (%)	0.3193	0.6037	-0.2063	-0.2722	-0.3409
Litter abundance (g m ⁻²)	-0.1705	0.0764	-0.0027	-0.4472	-0.4700
Litter decomposition (%)	0.6052	-0.1822	0.0677	0.0118	0.3342
Earthworms m ⁻²	-0.1029	0.3877	0.0211	0.2130	-0.1210
Infiltration rate (cm min ⁻¹)	0.5887	0.0828	-0.1573	-0.2888	-0.0880
Soil moisture (%)	-0.2466	0.0688	-0.2553	0.5623	-0.4195
pH	0.3985	-0.2647	0.7311	-0.1106	0.2493
Organic matter (%)	0.9571	0.1204	-0.0005	0.0269	-0.0105
TOC (ppm)	0.8862	0.0736	0.1826	-0.0077	-0.0011
CEC (meq 100g ⁻¹)	-0.1080	0.0438	0.4694	-0.3396	-0.0991
N (%)	0.9174	0.1175	0.0580	0.1866	-0.0061
P (ppm)	0.6783	-0.0334	0.1227	-0.1908	-0.1780
K (ppm)	0.4387	-0.3274	0.2316	0.5045	-0.1086
Ca (ppm)	0.4687	-0.1911	0.8280	0.0419	0.1042
Mg (ppm)	0.5009	-0.1475	0.8084	-0.0201	0.1584
Zn (ppm)	0.0093	-0.4733	-0.1332	-0.0308	-0.1077
Mn (ppm)	-0.2850	-0.2649	0.8027	0.1703	-0.2259
Cu (ppm)	-0.5721	-0.0717	-0.0783	0.6074	-0.1708
Fe (ppm)	-0.2402	-0.1506	-0.0933	-0.0331	-0.4664
B (ppm)	0.4955	-0.1305	0.8115	0.0588	0.1230

Supplemental Table 3. Unique resident plants within specific treatments. Same species are ordered by row.

Present in RE not IN
<i>Acalypha virginica</i>
<i>Actaea racemosa</i>
<i>Arisaema triphyllum</i>
<i>Athyrium filix-femina</i>
Unknown grass 1
<i>Carex swanii</i>
Unknown forb
Unknown tree
<i>Dioscorea villosa</i>
<i>Fatoua villosa</i>
<i>Galium circaezans</i>
<i>Geranium carolinianum</i>
<i>Hypericum canadense</i>
<i>Ligustrum sinensis</i>
Unknown rosette
Unknown grass 2
<i>Stellaria pubera</i>
<i>Osmunda claytoniana</i>
<i>Pinus strobus</i>
<i>Polygonum convolvulus</i>
<i>Prunella vulgaris</i>
Unknown rush
<i>Celastrus orbiculatus</i>
<i>Solidago</i> spp.
<i>Solidago curtisii</i>
Unknown grass 3
<i>Stellaria media</i>
<i>Viola</i> spp.
Unknown grass 4
<i>Uvularia grandiflora</i>
<i>Vaccinium angustifolium</i>
<i>Veronica officinalis</i>
<i>Viola hirsutula</i>
<i>Vitis</i> spp.

Supplemental Table 4. Magnitude and direction of differences between invaded and reference site (Δk) of individual EIMs without absolute values and separated by site.

Measured EIMs	Removed Reference			Uninvaded Reference		
	BBS	PAN	POT	BBS	PAN	POT
Diversity index (H')	-0.598	-0.655	-0.616	-0.559	-0.694	-0.643
Diversity index w/o invader	-0.182	-0.145	-0.155	-0.142	-0.260	-0.300
Richness	-0.205	-0.322	1.754	-0.151	-0.208	-0.101
Native richness	-0.236	-0.373	-0.375	-0.236	-0.400	-0.035
Bare ground (%)	-0.902	-0.966	-0.805	-0.900	-0.978	-0.919
Other invader cover (%)	0.000	-0.631	-0.712	0.000	1.375	-0.917
Native cover (%)	-0.183	-0.583	-0.474	-0.256	-0.140	0.914
Microbial biomass (mg kg ⁻¹)	-0.268	0.099	-0.305	-0.269	-0.118	-0.614
Microbial activity ($\mu\text{g CO}_2$ 10g soil ⁻¹ hr ⁻¹)	-0.209	-0.005	1.594	0.114	-0.069	0.807
Light penetration (%)	-0.478	-0.331	-0.636	-0.532	-0.564	-0.657
Litter abundance (g m ⁻²)	-0.108	0.013	0.266	-0.237	-0.046	-0.109
Litter decomposition (%)	0.919	0.110	-0.467	-0.084	0.198	-0.448
Earthworms m ⁻²	0.000	-1.000	-0.977	-1.000	-1.000	-1.000
Infiltration rate (cm min ⁻¹)	1.033	0.526	-0.425	-0.527	5.677	-0.797
Soil moisture (%)	0.013	-0.058	0.047	0.352	-0.007	0.228
pH	-0.006	0.006	-0.197	0.103	0.054	0.092
Organic matter (%)	0.033	0.024	-0.618	-0.061	-0.074	-0.758
TOC (ppm)	-0.203	-0.027	-0.571	0.138	-0.120	-0.693
CEC (meq 100g ⁻¹)	0.158	-0.153	0.196	-0.104	0.054	0.430
N (%)	-0.217	-0.025	-0.782	0.091	0.011	-0.800
P (ppm)	0.000	0.000	-0.204	0.000	0.000	-0.368
K (ppm)	0.063	0.203	-0.710	0.052	0.944	-0.670
Ca (ppm)	-0.006	-0.045	-0.723	0.267	0.938	0.023
Mg (ppm)	0.050	-0.023	-0.352	0.590	0.443	-0.314
Zn (ppm)	0.024	6.786	1.617	0.753	8.083	1.394
Mn (ppm)	0.041	0.221	0.834	0.010	0.314	1.894
Cu (ppm)	0.000	0.053	1.045	0.091	0.404	1.000
Fe (ppm)	0.196	0.009	3.700	1.769	-0.145	2.267
B (ppm)	0.000	0.000	-0.747	0.000	0.053	-0.400

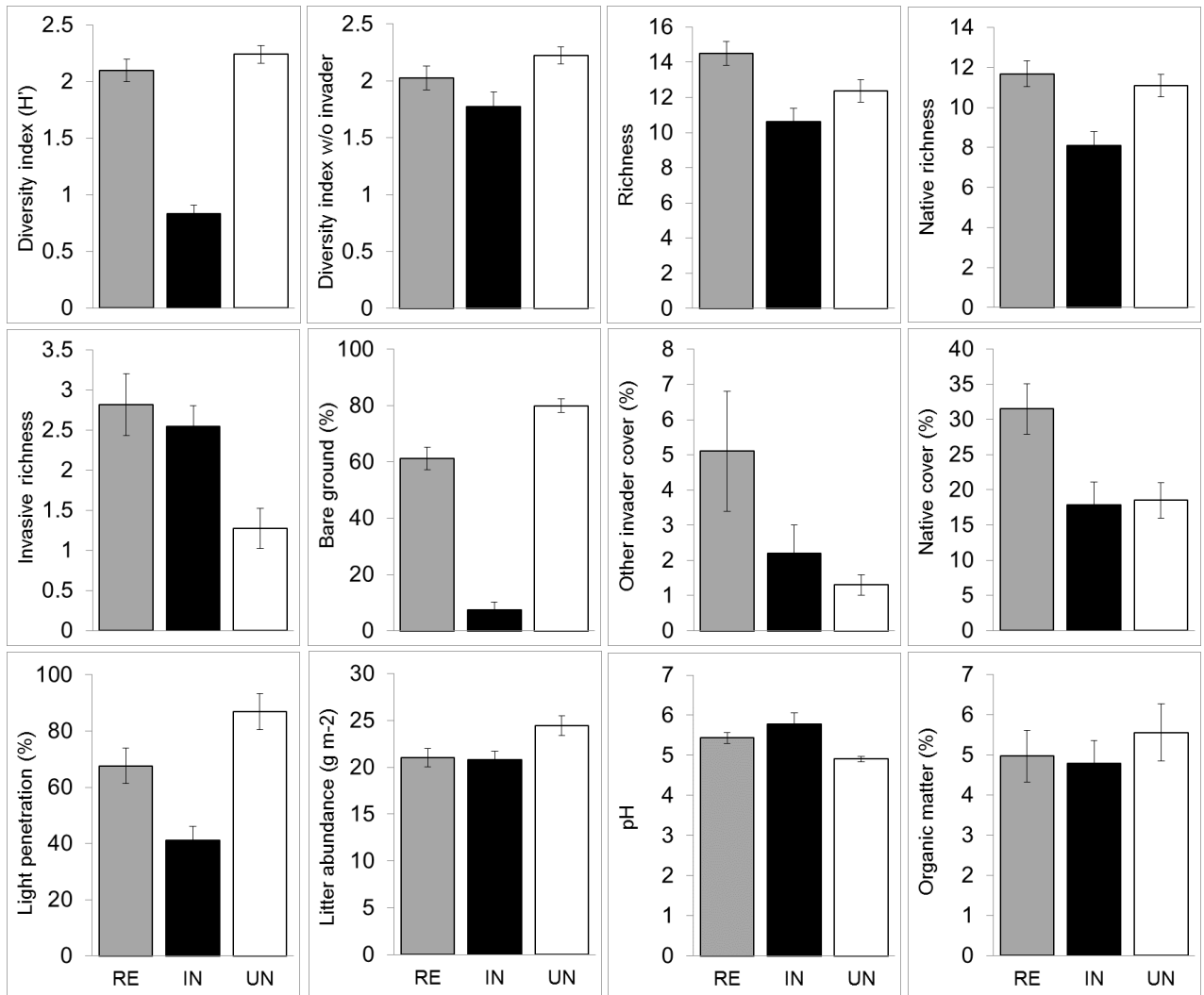


Figure 1. Univariate plots of significantly different EIMs in plant community metrics, other abiotic/biotic property metrics, and soil physical property metrics; grey bars are RE plots, black bars are IN plots, and white bars are UN plots. For nutrients results see Table 1 and 2.

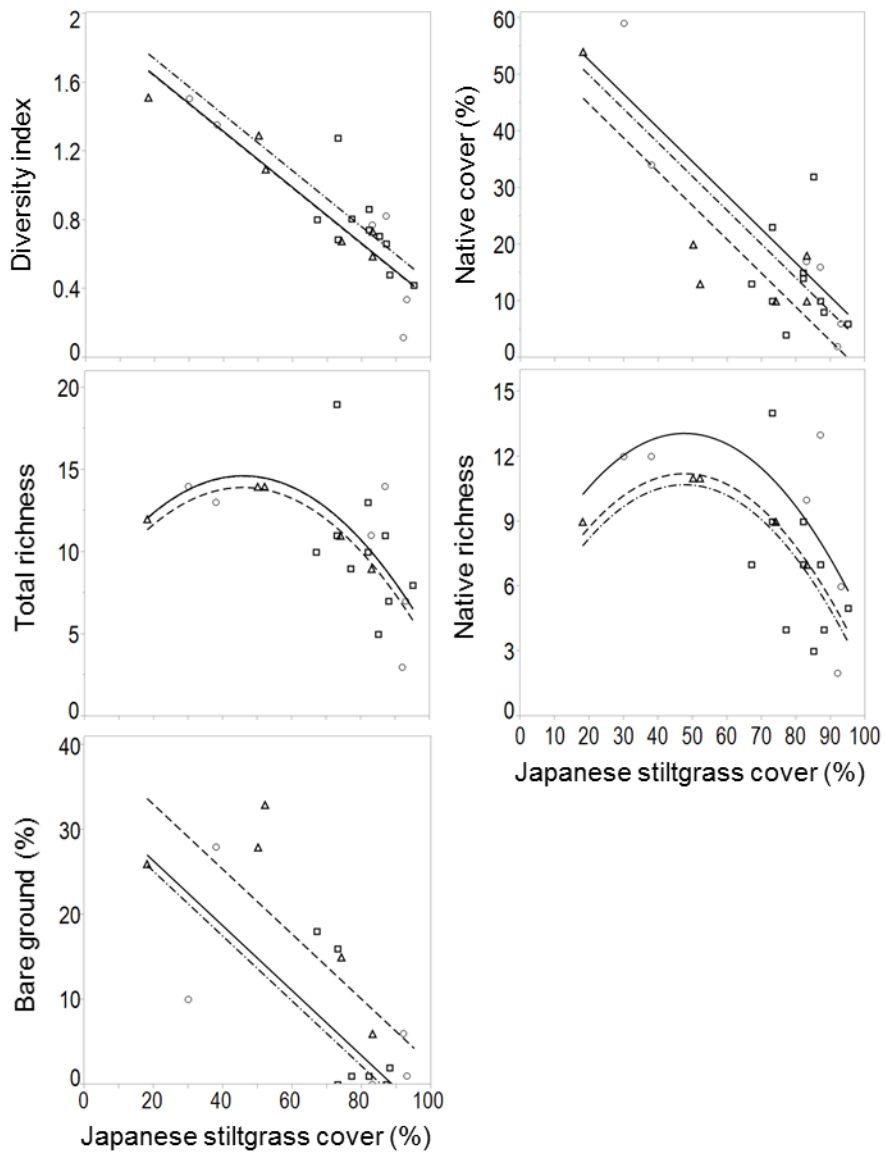


Figure 2. Significant ANCOVAs for univariate analysis of Japanese stiltgrass cover and EIMs. Circle and solid lines represent BS, squares and dash-dot lines represent PA, and triangles and dashed lines represent PO.

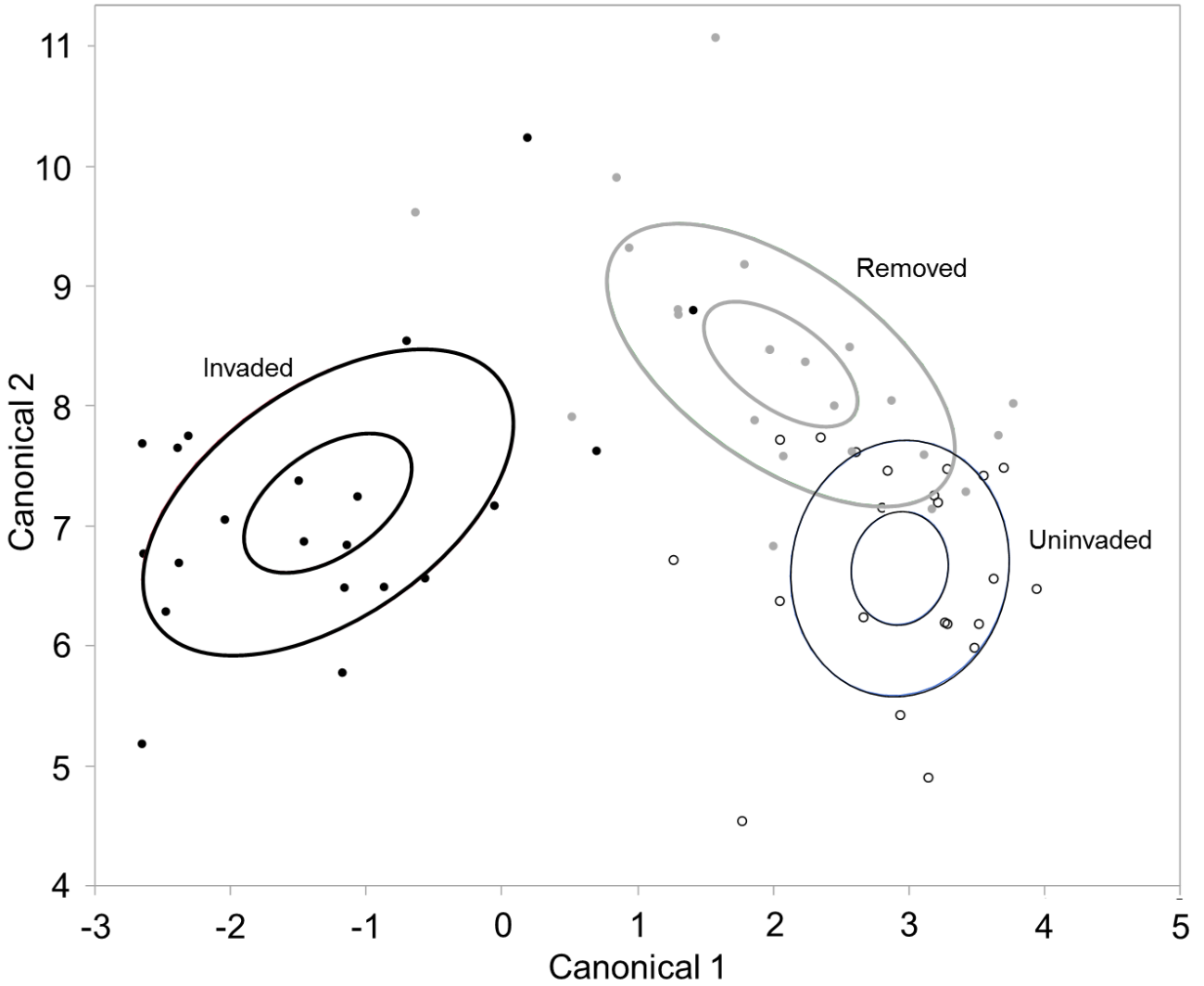


Figure 3. Quadratic discriminant analysis of flagship EIMs (organic matter, diversity index, pH, invasive richness, native cover) displaying canonical axes one and two. Black circles represent IN plots, grey circles represent RE plots, and white circles represent UN plots. Inner ovals represent 95% confidence of the treatment mean and outer circles represent the area required to account for 50% of points within the treatment group.

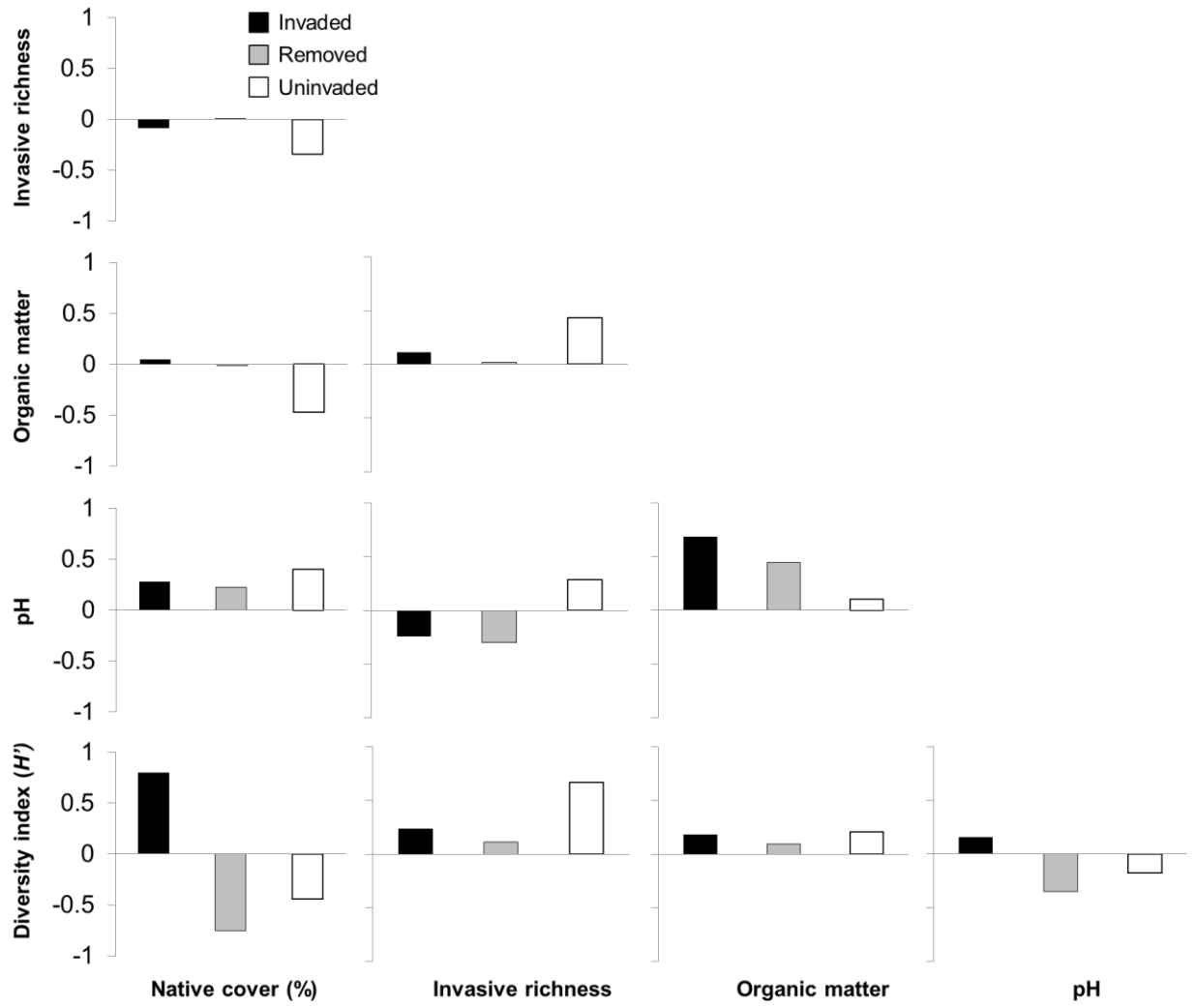
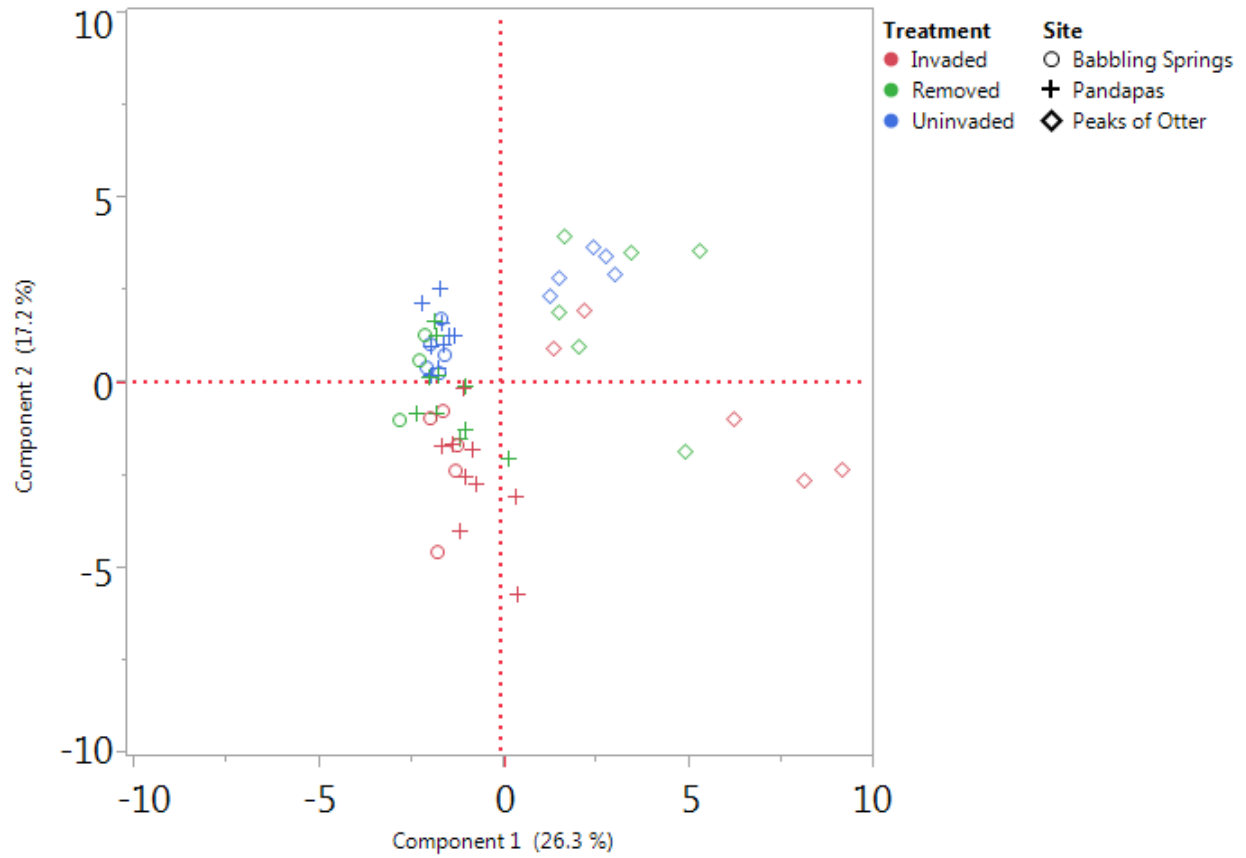
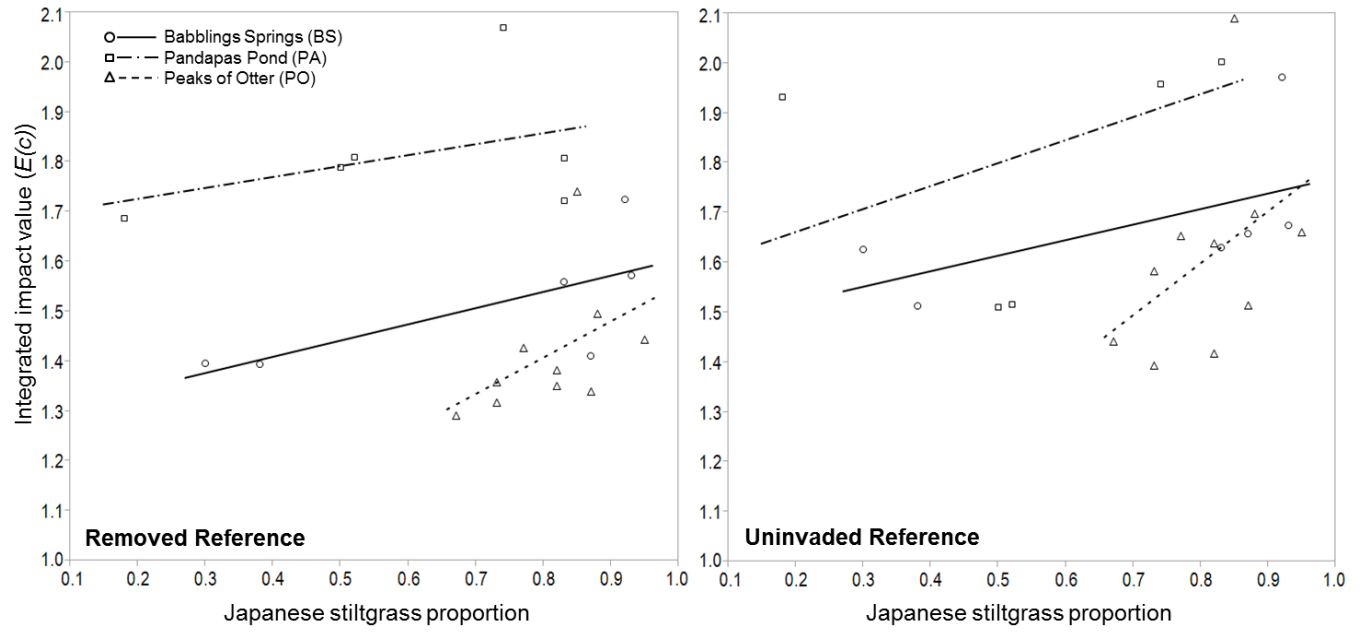


Figure 4. Correlation structure of flagship EIMs with bars representing correlation strength; black bars are IN plots, grey bars are RE plots, and white bars are UN plots.



Supplemental Figure 1. PCA of invaded, removed, and uninvaded plots. Invaded plots are red, removal plots are green, and uninvaded plots are blue. Babbling Springs plots are circles, Pandapas plots are crosses, and Peak of Otter are diamonds.



Supplemental Figure 2. ANCOVA of $E(c)$ values separated by site over the cover of Japanese stiltgrass.

Chapter 3: Impact shadows: the temporal effects of invasive plant establishment and removal

Abstract:

A diverse breadth of invasive plant impacts has been identified that include reductions in biodiversity, changes in biogeochemical cycling, and disruptions of ecosystem function. To mitigate negative ecological impacts, land managers target invasive plant removal often with the goal of local eradication. However, invader removal does not necessarily restore a site to pre-invasion ecosystem conditions, as invader impacts can persist as “legacy effects”. In fact, very little is known about the temporal effects of invasion impact, both following establishment and after removal. To identify invasion shadows (accrual and loss of invader impacts), we measured the changes in biotic and abiotic ecological parameters during invader establishment and following removal. We found that although individual ecological metrics do not show any clear pattern, when integrated, seeded areas became more similar to the invaded landscape and removed areas became more similar to the uninvaded landscape. The plant community did not respond in a three year period to seeding the invader, and the plant community became less similar to both the invaded and uninvaded landscape altogether when removals were performed. Surprisingly, no gradual temporal effects were seen in impact accrual or loss. Instead, changes occurred almost immediately and persisted, which is especially surprising following invader establishment. Our results show that some invader impacts accrue immediately following establishment, and that ecosystems do not return to an uninvaded state within three years after removal, and may trend further from the desired uninvaded goal. This has implications for management prioritization and our fundamental understanding of how invasive plants change ecosystem processes.

Introduction:

Invasive species are regarded as one of the top five current and ongoing threats to human and ecosystem welfare topped only by habitat degradation and resource over-exploitation (Millenium Ecosystem Assessment 2005). Invasive plants are reported to negatively impact nearly all aspects of ecosystems (Vilà et al. 2011), which in turn cost an estimated \$25 billion in damages annually in the United States alone through reductions in ecosystem services (Pimentel et al. 2005). Invasive plants can negatively impact native plant community composition (Pyšek et al. 2012), soil nutrients and microbial community (Liao et al. 2008), and ecosystem properties (Vilà et al. 2011), though this likely underrepresents the actual breadth and magnitude of invasive plant impacts, which are poorly known for most invaders (Hulme et al. 2013).

To ameliorate negative ecological impacts, tremendous resources are allocated for controlling, managing, and attempting to eradicate invasive plants (Myers et al. 2000). Although most landowners desire to permanently remove (i.e., eradicate) problematic invasive plants, in most cases eradication is not feasible for established invasive plants (Rejmánek and Pitcairn 2002). Therefore, invasive species management is an ongoing task to reduce local population sizes and limit seedbank recruitment. In any case, many invasive plants are managed in an attempt to restore ecosystem function that was altered by the invader.

When the invasive plant is believed to be the driver of ecological change (MacDougall and Turkington 2005), the offending invader is removed in a desire to return the system to a pre-invaded state, which is analogous with restoration (Corbin and D'Antonio 2012). Unfortunately, there is very little support of local eradication resulting in a restored ecosystem. Although restoration is most often a land management goal, as Corbin and D'Antonio (2012) explain, “even where local eradication is achieved, removal by itself is unlikely to allow restoration of

broader community or ecosystem characteristics.” This may be a function of the invasive plants not being the driver of change (MacDougall and Turkington 2005), or a vestige of the invasion persisting following removal.

Although the direct physical presence of the invader can be removed, residual impacts may remain. Instead of an instantaneous return to a pre-invaded ecological state, a “legacy” of the invader may persist and, thus, invader-mediated changes persist. For example, although a species that increases soil pH may have been locally removed, this increase in pH may remain long after the plant is removed. Legacy effects can vary in temporal persistence (Marchante et al. 2015), and may differ among aspects of an ecosystem (Cuddington 2011). In some cases, the legacy may also persist in perpetuity unless intervention is taken (Hacker and Dethier 2009, Hobbs et al. 2009). The persistence of legacy effects has obvious consequences for achieving restoration goals, but are as yet poorly understood for most ecological parameters for the majority of invaders.

In addition to the “backend” of invasion leading to potential legacy effects following invader removal, little is known about the accrual of impacts on the “frontend” during establishment of new invasive populations. In most cases invasive plant impacts are recorded on well established populations of unknown age, the results of which are applied broadly to populations of any age. However, as much as we have identified that some impacts scale with invader biomass (e.g., Pearson et al. (2015)), there is reason to believe that some impacts may scale with invasive population age. Impact accrual rates would greatly benefit management prioritization to target populations before peak impact, the rates of which likely vary among biotic and abiotic ecological parameters.

Temporal effects of invasive plant impacts are an important but often overlooked element of understanding the accrual and loss of impacts, which we term invasion shadows. We hypothesize that establishing invasive populations will accrue impacts slowly, thus casting a short shadow, while after removal invasive plants may continue to cast long impact shadows for some ecological parameters. Some studies have shown legacy effects in individual impact metrics, but no study to date has tried to look at potential legacy effects of both biotic and abiotic characteristics individually or together. In fact, few studies consider temporal effects at all, either on the front or backend of invasion. Here we determine if a common understory invader has any impact shadows on biotic and abiotic environmental characteristics, and if so, at what rate they accrue or diminish following invader removal.

Methods:

Three sites were selected across the Ridge and Valley section of Virginia as described in Tekiela and Barney (2015). The experimental design of Barney et al. (2015) was followed in accordance with the Global Invader Impact Network (GIIN) and described in Tekiela and Barney (2015), a summary of which follows. In 2013, at each location, four treatments were established; two within the invasion and two within the uninvaded site. Within the invasion we randomly located spatially paired 1.75 x 1.75m *invaded* (IN) and *removal* (RE) plots. IN plots were left un-manipulated, while RE plots were managed by hand pulling all *M. vimineum* individuals in June of 2013 to 2015, and then re-managed in July to remove any new seedlings. Within the uninvaded site, we randomly located *uninvaded* (UN) and *seeded* (SE) plots. UN plots were left un-manipulated to represent the forest understory vegetation as a reference control. SE plots were sown with *M. vimineum* seed at the same density of the surrounding invasion.

Data collection occurred in late July of 2013 through 2015, approximately at peak growing season, to capture the greatest influence of *M. vimineum*. Each vascular plant in each plot was identified to species and the percent groundcover was visually assessed to the nearest 1%. Five 1cm diameter by 10cm deep soil samples were collected in each plot and homogenized. Samples were then air dried, sieved with a 2mm sieve, and analyzed for plant available soil macro and micro nutrients, pH, and cation exchange capacity. Soil moisture was measured using an electronic soil moisture probe and soil infiltration rate was calculated using a 10cm diameter ring driven 10cm into the ground and infiltrated with 600ml of water. Additionally, light penetration was calculated by measuring photosynthetically active radiation (PAR) above and below the forest understory layer.

To test the effect of removals and seeding on individual ecosystem metrics and plant richness, an analysis of variance was run on each dependent variable individually using year (1, 2, or 3), treatment, site, and the interaction of treatment x year as fixed effects. A post-hoc Tukey's test was used to separate means.

We also wanted to compare the similarities (or differences) of plant communities among the treatments, which is not possible using standard richness, biodiversity, or similarity metrics. Similarity metrics can compare unique species among plots, but do not consider species abundance (Ricotta and Szeidl 2006). Invasive plants are known to modify the abundance of native species, but rarely cause extinctions (Gurevitch and Padilla 2004). Thus, we wanted to capture this change in abundances, which required developing a new method to estimate the similarity of community composition that is abundance explicit by describing the 'plant community distance' between each plot.

We created a matrix of all plant species and their cover in each plot excluding *M. vimineum*. This dataset was subjected to a principle component analysis that was varimax rotated to center, rotate, and orthogonalize the data. Then, a factor analysis was used to reduce complexity by removing any components with variance explained <1. This reduced the plant community from 148 species to 60 components. The same technique was applied to all soil and ecosystem property metrics, which was reduced from 15 ecosystem properties to 6 components.

For plant community and ecosystem analyses, the center of mass (COM) of IN and UN treatments was calculated independently for each year and site in multivariate space by averaging each treatments plots (i.e., IN and UN) across each component. Then, the Euclidian distance was calculated between every plot in each treatment and their respective IN and UN COMs (i.e., correct removal year and site) because these two are the ‘original state’ references and allow for comparison of each treatment to the pre-seeding and removal state. Analysis of variance was performed on log transformed Euclidian distances of each treatment to the COM of IN and UN plots with sums of squares partitioned by removal year, treatment, site, and the interaction treatment x removal year and a post-hoc Tukey’s test with $\alpha=0.05$ was used to separate Euclidian distance means.

Results:

For individual ecological metrics, the treatment-by-year interaction was never statistically significant, suggesting the patterns among the treatments remained consistent following establishment and removal (Table 1). However, treatment means were different for all independent ecosystem metrics except CEC, Fe, and soil infiltration (Table 1). Of those metrics, IN and UN means were different for all metrics except soil infiltration rate. Of these, pH, CEC,

P, K, Zn, Cu, Fe, soil infiltration, and soil moisture were similar in IN and RE plots, while Ca, Mg, Mn, B, and light penetration did show a significant difference between IN and RE plots (Figure 1). Only Zn showed a difference between IN and SE plots (Figure 1).

When the ecological metrics were integrated and their distances measured, there were large differences among treatments compared with the UN reference, and less so when compared to the IN reference (Figure 2a & b). The IN plots were furthest from the UN reference with SE and RE plots at middle distances (Figure 2a and Table 2). The distances of the UN and SE were significantly further from the IN compared with the UN across all three years but RE was not (Figure 2b), with no treatment x year effect (Table 2).

The patterns for plant community distances were less straightforward than the ecological metrics (Figure 2c & d). When the resident plant community distances were compared to UN, the RE plots were furthest, while the SE and IN were similar to UN (Figure 2c), with no treatment x removal year interactions (Table 2). When compared to IN, in general the UN, SE and RE plots were similar to each other and significantly separate from IN (Figure 2d).

Plant richness also did not have a significant treatment x year interaction term, with RE plots having the greatest richness, while IN and SE had lower richness than UN (Figure 1). There were no richness changes over the duration of the experiment.

Discussion:

In nearly all cases single ecological metrics were affected by the presence of the invader *M. vimineum*. Thus, the invaded plots had significantly different values than uninvaded plots. In other words, the invader is having an impact broadly, which has been corroborated by many studies of this species across a range of geographies (e.g., Kourtev *et al.* 1998, 2003; Ehrenfeld

et al. 2001; McGrath & Binkley 2009; Tekiela & Barney 2015). Overall, the impacts of *M. vimineum* accrued quickly following establishment and remained following removal, suggesting a temporal impact in both directions.

Impact legacies

Following three years of removal, with no *M. vimineum*, more than half of the abiotic ecological metrics remained similar to the invaded plots. However, of the metrics that did change following removal, they changed immediately, not slowly returning to an uninvaded state as predicted. Furthermore, although the removal plots shifted in the direction of uninvaded plots generally, only P, Mn, and light penetration were similar to uninvaded plots after three years of removals. However, when the abiotic ecological impacts were integrated, much of the individual metric noise was reduced, and a clear pattern emerged. Removal plots became more similar to uninvaded than invaded plots, but did not achieve an “uninvaded state”. This immediate change followed by persistent legacy effects was also shown in *Cytisus scoparius*, and was suggested to be due to the nitrogen pulse left by the carcasses of managed plants, however this *C. scoparius* is a nitrogen fixer (Grove et al. 2015). However, in our study *M. vimineum* was hand managed and all biomass was removed thus, these dramatic litter legacy effects could not exist. Although we did not examine the soil microbial community, long lasting legacies in the microbial community (e.g., Elgersma et al. 2011, König et al. 2015) could have changed the nutrient cycling characteristics of this system and been responsible for these long lasting soil legacies that did not appear to change in three years. In short, removing the invader made the abiotic components of the ecosystem become more associated with an uninvaded state, but more time would be needed to identify if removing the invader returned it to an “uninvaded state”.

Plant richness was greatest in removal plots, surprisingly more so than uninvaded plots. This may result from the intrinsic disturbance from removing the invader, though this was relatively minor as plants were removed at the seedling stage. Not only does the removal create a minor soil disturbance, but it also modifies the light exposure to the soil surface which has been shown to effect germination (Baskin and Baskin 1998). Many of the species that emerged in removal plots were weedy species themselves (e.g., *Polygonum persicaria*, *Pilea pumila*) which may have responded to the disturbance, and may partly explain this unexpected increase in richness.

Overall, the resident plant community showed minor compositional changes due to the presence of *M. vimineum*. In fact, removing *M. vimineum* may have done more harm than good and further distanced the resident plant community from an uninvaded or invaded landscape. The resident plant community became entirely novel from both reference treatments. In this case, other ruderal weedy species that were not well represented in either IN or UN plots now dominated following removal. In context of legacy effects, this is a worst-case scenario, as the legacy in fact makes the new “restored” plant community even less like the uninvaded landscape. This negative response to management is often seen in Western United States systems when the removal of invasive perennial forbs does little to restore the native plant community, and instead encourages establishment of new invasive annuals grasses (Skurski et al. 2013). This is additionally concerning for *M. vimineum* invasions, because re-establishment of this invader is also likely, potentially leaving an even less similar community when management is terminated (DeMeester and Richter 2009). If a return to an uninvaded state is desirable, simply removing the invader, for three years at least, is inadequate. Rather, active restoration involving reseeding of desirable species may be the only viable option (Herget et al. 2015).

The legacy effects we observed may be partially due to the ongoing management of *M. vimineum* that was required for the duration of this study. Invasive plants are almost never eradicated with one or few years of management due to seed banks or perennial regeneration through resprouts or rhizomes. Plant management itself is a disturbance that may obscure parsing impacts, and can cause negative externalities on the ecosystem such as reduced native plant cover (Rinella et al. 2009) and establishment of new invaders (Skurski et al. 2013). Nevertheless, if this study were continued until management was no longer required (i.e., the invader was eradicated), the results may be different and are likely to change with time (Dostál et al. 2013). Obviously this study is constrained to only the first three years after initial removal; therefore, we were not able to identify longer-term changes in legacy effects. Nevertheless, this highlights the importance that management must be a long-term investment, and that restoration will be best achieved through active replanting of desirable plants in addition to invader management.

Accruing impact

The accrual of ecological impacts following a new invasion is very poorly understood, yet has important implications for our broader understanding of how invasive plants interact with their ecosystem, and would aid management prioritization to small populations that accrue impacts quickly. In our study, when looking at individual ecological metrics, there was little evidence for impact changes following invader establishment. In other words, most metrics were not different from uninvaded levels. Only a single micronutrient (Zn) shifted towards an invaded state. Importantly, seeded plots remained at very low invader density for the first two years (~1%), and only began to establish in the 3rd year with ~20% cover, which is much lower than

older established *M. vimineum* invasion at 80-100% (Tekiela and Barney 2015). For many invasive plants, their ecological impacts are proportional to their abundance (i.e., cover, density), and non-linear relationships have been identified in many species (Thiele et al. 2011, Tekiela and Barney 2015). In fact, *M. vimineum* has been shown to begin impacting some ecological factors at >40% cover, which was not achieved in this study (Tekiela and Barney 2015). Thus, our results should be viewed within the context of the relatively low cover achieved within three years of introduction.

Once integrated, the abiotic metrics painted a different picture than when viewed individually. The seeded plots immediately began to differentiate from an uninvaded state, and were intermediate to the removed plots. Intriguingly, these shifts happened within the first year following introduction, and then we saw no additional changes.

One year after initial introduction, the seeded plots had reduced species richness compared to the uninvaded plots, but we did not observe an overall change to the resident plant community in three years. This lack of overall differences is reflected in the equal weighting of changes to species richness and cover in our analysis. Richness remained similar to the uninvaded resident plant community and dissimilar to the invaded plant community; though this may not be surprising considering the *M. vimineum* cover was so low and its residence time short. Similarly, Dostál et al. (2013) showed that newly established giant hogweed (*Heracleum mantegazzianum*) imposed greater reductions in species richness early in the invasion process, but their time frame was much longer and their initial *H. mantegazzianum* had greatest cover early on in the invasion, further suggesting this is likely a function of cover not time.

Overall, we found temporal effects do exist on both the backend (following removal) and frontend (following introduction) of invasion, though they likely function at different time scales. Many of the observed changes occurred immediately after introduction or removal, which has important implications for how we view early invasions and managing invaders to achieve restoration. Most concerning from a management perspective is the shift of the resident plant community to a novel condition not seen in either the invasion or when uninvaded. If management efforts only replace a problematic invader with other weedy species, the cost of management may outweigh its gains. The mitigation of ecological impacts is not as straightforward as removing the invader, and much could be learned from longer-term studies that follow invader management and the concomitant changes to the ecosystem.

Acknowledgements: We would like to thank VTIFE lab for all their assistance with field work.

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Table 1. Statistics from ANOVA of individual metrics. . See supplemental figure 1 for means.

	Year			Treatment			Treatment x Year		
	DF	F-value	p-value	DF	F-value	p-value	DF	F-value	p-value
pH	2	1.86	0.1586	3	28.04	0.0001	6	0.54	0.7804
CEC	2	6.78	0.0014	3	0.87	0.4577	6	0.41	0.8754
P	2	1.80	0.1683	3	6.66	0.4577	6	0.83	0.8754
K	2	8.11	0.0004	3	15.38	0.0001	6	1.28	0.2657
Ca	2	0.415	0.6605	3	23.07	0.0001	6	0.26	0.9571
Mg	2	0.55	0.5788	3	17.44	0.0001	6	0.13	0.9920
Zn	2	2.78	0.0640	3	7.07	0.0001	6	0.62	0.7065
Mn	2	2.59	0.0771	3	8.37	0.0001	6	1.35	0.2374
Cu	2	22.30	0.0001	3	12.68	0.0001	6	0.66	0.6817
Fe	2	0.72	0.4869	3	1.66	0.1766	6	0.35	0.9069
B	2	0.14	0.8659	3	12.32	0.0001	6	0.09	0.9971
Soil infiltration	2	4.15	0.0168	3	0.19	0.9065	6	0.48	0.8235
Soil moisture	2	172.37	0.0001	3	5.29	0.0015	6	0.71	0.6413
Light penetration	2	0.93	0.3961	3	20.65	0.0001	6	2.00	0.0654

Table 2. Statistics from ANOVA of integrated metrics comparing distance of plots to multi-dimensional center of mass (COM) of invaded (IN) and uninvaded (UN) plots. . See Figure 2 for distance to COM means.

	Year			Treatment			Treatment x Year		
	DF	F-value	p-value	DF	F-value	p-value	DF	F-value	p-value
To IN COM									
Resident plants comm.	2	9.44	0.0001	3	23.75	0.0001	6	0.70	0.6506
Ecosystem properties	2	12.64	0.0001	3	15.16	0.0001	6	0.83	0.2294
To UN COM									
Resident plants comm.	2	9.03	0.0002	3	14.25	0.0001	6	0.86	0.5232
Ecosystem properties	2	0.63	0.5344	3	46.35	0.0001	6	0.13	0.3304

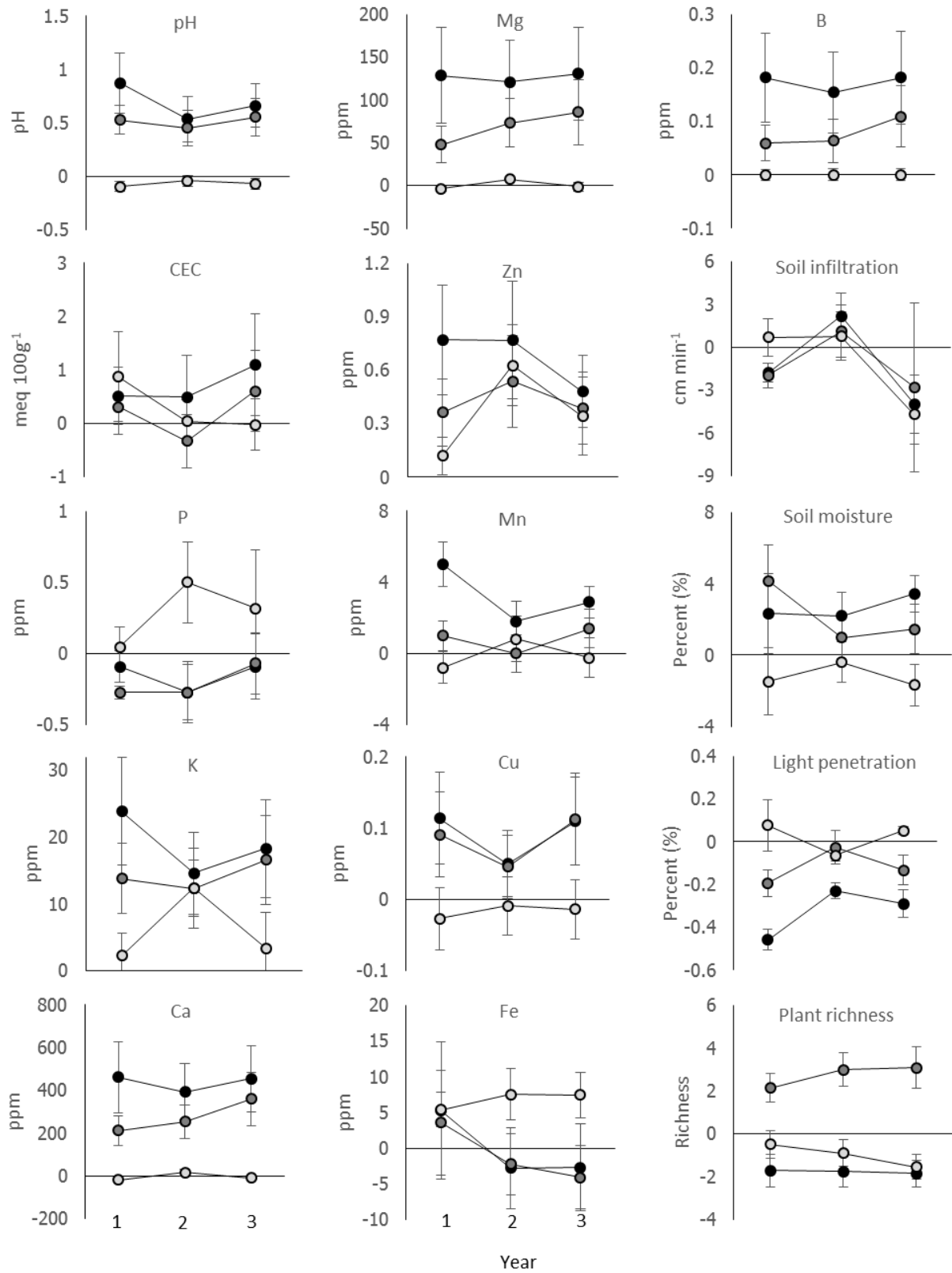


Figure 1. Difference between (Δ) individual metrics of invaded (IN, black circles), removed (RE, dark grey circles), and seeded (SE, light grey circles) plots compared to uninvaded (UN) plots for each year after initial removal and seeding.

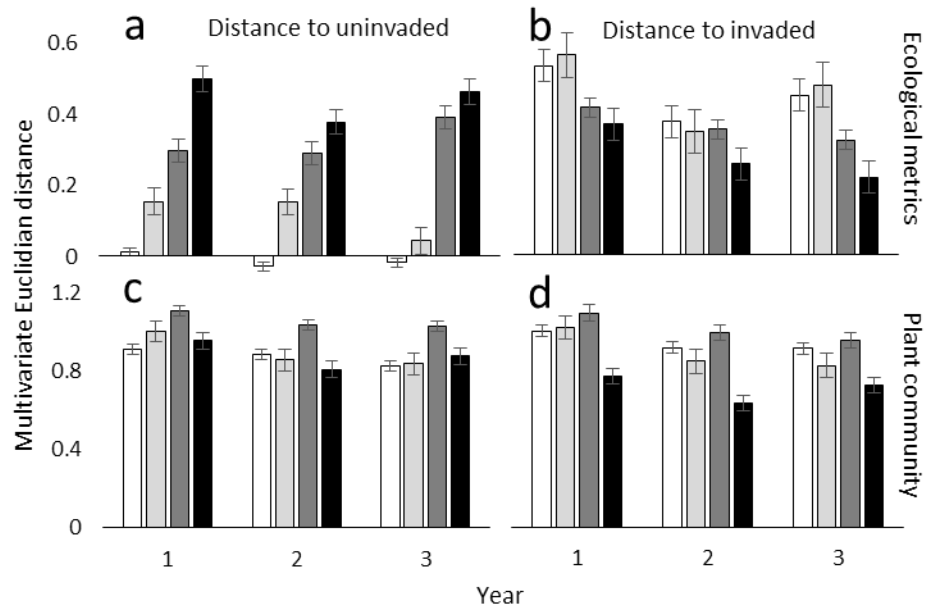


Figure 2. The Euclidian distances to the multi-dimensional center of mass (COM) of both uninvaded (UN, a&c) and invaded (IN, b&d) plots for each year for ecological metrics (a&b) and the plant community (c&d). UN plots are white, seeded (SE) plots are light grey, removal (RE) plots are dark grey, and IN plots are black. Greater distances suggest less similarity to the respective COM.

Chapter 4: Co-invasion of similar invaders results in analogous ecological impact niches and no synergies

Abstract:

Exotic species can cause changes to their invaded ecosystems, which can be large and long lasting. Despite most landscapes being invaded by multiple exotic plant species, >90% of impact studies only characterize the impacts of single species. Therefore, our knowledge of invasive plant impacts does not reflect the co-invaded nature of most landscapes, potentially ignoring complex interactions among exotic species. Our objective was to characterize potential synergies (positive interactions) on biotic and abiotic ecological parameters among the important forest invaders Japanese stiltgrass (*Microstegium vimineum*) and wavyleaf basketgrass (*Oplismenus undulatifolius*), which co-invade eastern US deciduous forests. To characterize synergies, we used a factorial selective removal study, as well as an observational study to further explore invader cover-impact relationships. Although both invaders can reduce native plant richness by 70% individually or in combination, there were no impact synergies. Total cover of any combination of the two invaders had a negative quadratic effect on total, exotic, and native plant richness; i.e., all community metrics were greatest at intermediate levels of total invader cover and lowest at maximum invader cover. Native richness was more greatly affected by the co-invasion than exotic richness. Soil metrics had no clear trend in either study. Japanese stiltgrass and wavyleaf basketgrass appear to have overlapping impact niches—the number, magnitude, and direction of biotic and abiotic changes to the invaded ecosystem—that only vary in impact magnitude, not breadth. As a result of their overlapping impact niches and non-synergies, in this co-invaded system, the addition of the recent invader wavyleaf basketgrass has not resulted in additional changes to the invaded forests. Future impact studies should focus on multiple species

and identifying synergies, especially as they relate to invader cover, which informs ecological interactions and management prioritization.

Introduction:

Invasive plants are of conservation concern due to the ecological impacts they impose on the ecosystems they invade (Blackburn et al. 2014, Jeschke et al. 2014). Thus, there has been a recent spotlight on quantifying these impacts (Vilà et al. 2011, Pyšek et al. 2012). Impact studies currently represent nearly 30% of all invasion literature (Lowry et al. 2013). Examples of ecological impacts attributable to invasive plants include reductions in native plant abundance, richness, and diversity (Vilà et al. 2011, Pyšek et al. 2012), changes in soil nutrient pools and cycling (Ehrenfeld 2010), and changes in trophic dynamics (Litt et al. 2014).

Despite this wealth of new information, the vast majority of invasive plant impact studies only examine the ecological impacts of single invaders (Vilà et al. 2011, Pyšek et al. 2012). In fact, only one third of existing studies even mention multiple invasive plants; and of that third, only 19% (a total of 6% of all invasive plant impact literature) explicitly examine how impacts between single and multiple invasive plants might differ (Kuebbing et al. 2013). In contrast, 69% of conservation projects managed within the global Conservation Project Database that are aimed at restoring degraded landscapes, list multiple invasive plants in their management planning; indicating co-invasion (>1 locally sympatric invasive plants) is far more commonly observed than studied (Kuebbing et al. 2013). In other words, land managers are more commonly faced with multiple invasive plants, while 94% of existing research precludes any understanding of these co-invasions (Kuebbing et al. 2013). Though studying the impacts of a single species is a good start, it does not meet the needs of land-managers and conservationists, nor does it reflect

the potential interactions among co-invading species. This disconnect will likely be further amplified by the predicted future rise in the rate of invasive species introductions (Cohen and Carlton 1998), which will further increase the likelihood of plant co-invasions.

One attempt to describe the ecological impact of multiple invasive plants is the additive/non-additive impact framework (Kuebbing et al. 2013). This framework explores the simplest co-invasion, a two-invader system. It suggests there are two primary types of interactions: the ecological impacts could be either additive ($1+1=2$) or non-additive ($1+1\neq 2$). Alternatively, Ricciardi et al. (2011) presented multiple relationships between the number of established invasive species and the ecological impact they impose. These relationships ranged from positive to negative and linear to asymptotic—although these relationships have never been empirically tested. Though informative, as will be discussed, neither example considers the complexities of invader cover or density, which has been shown to influence impact magnitude in several cases (e.g., Gooden et al. 2009; Greene and Blossey 2011).. In fact, we argue what is described as additive in these frameworks may be considered a synergy due to the importance of cover.

Different invasive plants can have very different or similar types of ecological impacts. For example, some invaders may be more likely to impact nutrient dynamics (e.g., nitrogen fixers), while others may be more likely to alter hydrology or native richness. Here, we call the breadth (i.e., number of ecological impacts) and magnitude (i.e., strength of ecological impacts) of biotic and abiotic changes attributable to one invader its *impact niche*. The combined ecological impact of co-invasions can have several possible relationships that can vary across the range of invader cover depending on the impact niches of individual invaders (Fig. 1 & 2). In a two species co-invasion, invaders can have completely independent or completely overlapping impact niches (Fig. 1a and c respectively). When impact niches are independent, the overall impact to the

system is merely a combination of all the independently impacted ecosystem metrics; synergy is not possible (Fig. 1a). When impact niche overlap occurs, the resulting impact can be synergy or non-synergy. Non-synergy exists when the magnitude of impact within the overlapping impact niche region is simply the average of the two invaders at any particular cover (Fig. 1).

Alternatively, synergy exists when the magnitude of impact in the region of overlapping impact niche is greater than the average of the two invaders. For example, if the two invaders equally reduce plant community richness, but impact a completely different suite of species, then we would expect that the co-invasion would have synergistic impacts that more greatly reduce plant community richness in community than independently (Fig 1c). Additionally, impact niches do not have to completely overlap or be completely independent and may only share some of the impact niche (Fig. 1b).

Identifying the impact of multiple invaders is complicated by invader cover-impact relationships. Even in single dominant invader systems, the magnitude of ecological impacts has been shown in many cases to vary with invader cover (Thiele et al. 2011, Tekiela and Barney 2015) i.e., the magnitude of impact is not the same across the range of possible invader covers. Though only 15% of current impact literature considers a range of the cover of the invader (Tekiela unpublished data); in nearly all studied cases, plant community metrics varied with invader cover (e.g., (Greene and Blossey 2011)). However, to our knowledge no studies examine invader cover-impact relationships of multiple invaders in co-invasion. As previously stated, synergy occurs when the impact of the invasive species in community is greater than their average *at the same total invader cover* (Fig 2a). This is important because the cover of the invaders can be non-independent if they exist in the same stratum (e.g., understory grasses)—for example, if invader 1 is 50% cover, the other invader can typically be 50% at most. The correct

comparison between two species that exist at 50/50% cover would be each species independently existing at 50% cover *not* at %100 cover (Fig 2a). Therefore, three cover values are potentially relevant; the total invader cover (invader 1 + invader 2, etc), as well as the proportion of total invader cover for each species (e.g., invader 1/total invader cover; Fig. 2b). If examined across invader proportional cover, non-synergy would be presented as a straight line, while synergy would be presented as any non-linear relationship (Fig. 2b). The cover-impact relationship of these three parameters will depend on a variety of issues, including whether the invaders impose similar impacts in a given ecosystem.

Our goal was to use this co-invader cover-impact framework to examine possible synergies among a variety of biotic and abiotic ecosystem parameters in the similar species JS and WB invasion. Specifically our objectives were 1) to determine if ecological impact synergies exist, and 2) test how total and proportional invader cover influences the co-invasion impact. In other words, are two invaders of similar life history more impactful than one, and if so how?

Methods:

Study System:

We used two complementary studies to address our objectives using the co-invading forest understory species Japanese stiltgrass (*Microstegium vimineum*) and wavyleaf basketgrass (*Oplismenus undulatifolius*). The long established invader Japanese stiltgrass and the very recent (i.e., <30years) invader wavyleaf basketgrass have begun co-dominating the forest understory of Eastern United States forests (Beauchamp et al. 2013). Japanese stiltgrass (JS) is an annual C₄ grass native to Asia that established >100 years ago in Tennessee (Fairbrothers and Gray 1972). Though JS can successfully invade high light areas such as along forest edges, its ability to

establish in deep shaded forest understories has allowed it to cover >260,000 hectares in the deciduous hardwood forests of the Eastern United States (Kuppinger 2000). The ecological impacts of JS are relatively well-studied (Tekiel and Barney 2015), with reported alterations to the plant community (Flory and Clay 2009, DeMeester and Richter 2010, Brewer 2010), soil microbial community (Kourtev et al. 2003), soil chemistry (Kourtev et al. 1999, McGrath and Binkley 2009, Strickland et al. 2010), and animal community (Baiser et al. 2008, Marshall and Buckley 2009, Simao et al. 2010).

Wavyleaf basketgrass (WB) is a perennial C₃ grass thought to be native to Europe and Asia that primarily invades shaded forest understories (Beauchamp et al. 2013). Unlike JS that is a long established invader in the United States, WB is a relatively new invasive species of the Eastern United States. Thought to be transported through the Baltimore, Maryland port, WB has become a rapidly spreading regional invader in <30 years (Beauchamp et al. 2013). Although currently only present in Maryland, Washington D.C., and Virginia, its short residence time suggests it may not have spread to its range limits, and will likely spread across much of the Eastern United States (Beauchamp et al. 2013) similar to JS. However, unlike JS, little is known about the ecological impact of WB. However, WB cover has been correlated with reduced resident plant community richness and increased biodiversity (Beauchamp et al. 2013).

Within the current WB range, JS and WB commonly co-occur as the dominant invaders (Beauchamp et al. 2013). Co-invading JS and WB occur with low to high total cover (JS + WB), as well as different proportions of each. Therefore, this is an ideal system to study the cover-impact relationships of two important invaders as well as identify possible impact synergies in a co-invasion that presents current and future management challenges.

The study was conducted 35 km northwest of Baltimore, Maryland on the eastern edge of Liberty Reservoir, which is located <5 km from what is thought to be the initial WB invasion (personal communications Kerrie Kyde, MD DNR). This temperate deciduous forest has a canopy predominantly composed of white oak (*Quercus alba*), red oak (*Q. rubra*), red maple (*Acer rubrum*), eastern cottonwood (*Populus deltoides*), and American beech (*Fagus grandifolia*).

Invader manipulation:

In the first study we used the standard practice of invader removal to identify impact relative to an adjacent similar un-invaded site (Tekiel and Barney 2015, Kumschick et al. 2015). Within a homogenous JS and WB co-invasion, we imposed a factorial removal comprising the following treatments: 1) invaded (+WB/+JS), which was left un-manipulated; 2) both removed (-WB/-JS), which was managed by selectively hand clipping JS and WB at the soil surface to achieve ~0% cover; 3) Japanese stiltgrass removal (+WB/-JS), where JS was selectively removed, 4) wavyleaf basketgrass removal (-WB/+JS), where WB was selectively removed, and 5) un-invaded (UN), which has no site history of either JS or WB presence but is adjacent to the co-invasion. In 2013, 10 full blocks were randomly located within the co-invasion (treatments 1-4) and adjacent un-invaded area (UN). Within the co-invasion each block comprised four 1.75 x 1.75 m plots with the outer 0.25 m perimeter serving as a border to reduce edge effects. Removals were performed in early June when invaders were small and at the one to two leaf stage and again in early July to remove new seedlings. We recognize that removing the invader presents a minor disturbance to the system; nor do we necessarily expect the -JS/-WB plots to function as the UN site. Additionally legacy effects could hinder the usefulness of -JS/-WB plots

(Barney et al. 2015). Therefore, we performed two years of removal which is greater than half of removal studies (Stricker et al. 2015). Despite these limitations invader removal remains one of the primary tools to identify invader impact (Kumschick et al. 2015), and when combined with the second complementary study below, we gain a more robust picture of the role of these two invaders.

During peak growth when both species have already effectively carpeted the understory and prior to most plants flowering in late July we recorded percentage cover for every plant species to the nearest 1% and their nativity status, collected three subsamples of the photosynthetically active radiation (PAR) above and below the invader canopy on an overcast day, and collected 0.0005 m² x 0.1 m deep soil samples using a standard soil corer and submitted soil samples for analysis of pH, organic matter, and plant available P, K, Ca, Mg, Zn, Mn, Cu, Fe, and B (see Barney et al. 2015). All data were collected within the center 1 x 1m of each plot. The same treatments were applied and data recorded in 2014 on the same plots.

Cover-impact relationships:

Others have shown that the magnitude of some ecological parameters varies with invader cover/abundance (Thiele et al. 2011, Barney et al. 2013). However, no studies have examined the range of covers of two species co-invading the same system. Because our cover data for both these species was not independent (Total cover = \sum individual plant cover, Fig. 3a) due to existing in the same stratum, we converted the invader cover data to total invader cover ($C_T = C_{js} + C_{wb}$) and proportional invader cover (C_{js}/C_T or C_{wb}/C_T) which was found to be independent and well distributed (Fig. 3b). Depending on the impact niche of each invader, the total invader cover ($C_T = C_{js} + C_{wb}$) and/or the proportion of each invader (C_{js}/C_T or C_{wb}/C_T) may differentially

drive ecological impact. The manipulative study above was not designed to address invader cover-impact relationships, so we implemented a second study to explicitly address invader cover on plant and soil parameters.

We surveyed a >16 ha section of the same co-invaded forest in July 2014, and haphazardly identified 160 1m² plots that met our criteria of spanning a range of low to high total invader cover (C_T) that varied in their relative proportion of each species (C_{js}/C_T and C_{wb}/C_T). This resulted in plots with C_T from 0 to 100%, representing C_{js} and C_{wb} ranging from 0 to 95% and 0 to 92% respectively (Fig. 3). This allowed us to span the range of covers of both invaders at various combinations with large replication to identify potential synergies between the two species. We collected the same resident plant community and soil data as above. Although other invasive plants existed in a proportion of plots, none ever existed in greater than 10% cover, which does not represent the typical spectrum of cover they exist in these ecosystems. Therefore we did not include the other invasive plant species as dominant invaders in the analyses.

Data analysis:

In the manipulative study, the plant community metrics of resident plant richness (all species excluding JS or WB), native plant richness, other invasive plant richness, resident community biodiversity (Shannon-Weiner H'), and soil nutrient parameters were separately subjected to an ANOVA within each year with treatment and block as factors, and means were separated using a post-hoc Tukey's honestly significant difference test. Resident community biodiversity was box-cox transformed to meet statistical assumptions prior to analysis and all other metrics were normally distributed and left untransformed.

In the observational study, to test if light availability was driving JS and WB cover, we ran linear regressions of C_T and C_{js}/C_T (we did not include C_{wb}/C_T as this is simply $1 - C_{js}/C_T$ and is thus not independent) against understory canopy PAR (i.e., light accessible to understory community). Forest canopy transmittance was not significantly different across the independent variables C_T ($p = 0.7795$) or C_{js}/C_T ($p=0.3310$) suggesting there was not a bias in the overstory during plot selection; there was no difference in the tree canopy among the plots. Multiple regression analysis was then performed of C_T and C_{js}/C_T on resident plant richness, native plant richness, invasive plant richness, biodiversity, and soil nutrients. To interpret the potential synergies of JS and WB, a quadratic term was included for both C_T and C_{js}/C_T . A significant C_{js}/C_T quadratic term would suggest synergy of JS and WB in co-invasion: negative synergy (positive quadratic term) or positive synergy (negative quadratic term) effects. Therefore, the multiple regression was performed using the linear and quadratic terms of the independent variables C_T and C_{js}/C_T and the interaction of their linear terms. Plant biodiversity, Mn, and Zn were transformed to meet statistical assumptions of normality. To identify the species uniquely or more greatly impacted by JS or WB, a logistic regression was performed across C_{js}/C_T using presence/absence of species in each plot and a likelihood ratio test to test for significance. Species were first selected based on their presence in uninvaded plots (plots with 0% JS and WB) and then invaded plots. Rare species (present in $\leq 10\%$ of plots) and very rare (present in $\leq 1\%$ of plots) were grouped for analysis. For species present in the uninvaded plots, eight were in cover $>10\%$; for species present in all plots, twenty four were in cover $>10\%$. Species were then grouped into the functional groups ferns, forbs, grasses, herbaceous vines, woody vines, sedges, and trees/shrubs and the same logistic regressions were run.

Results:

Invader manipulation:

Removals were successful both years at reducing the target invader to near 0% cover. The WB in the +WB/-JS treatment responded positively to the removal of JS and increased from 57% to 78% cover in 2014 (which is representative of the area). However, JS cover in -WB/+JS only increased from 16.8% to 21% which is ~60% lower than a typical heavily invaded Japanese stiltgrass monoculture in this area (Fig. 4).

All plant community responses and their magnitudes were statistically similar in 2013 and 2014; therefore, only results from 2014 are reported. Total resident plant richness was two-fold greater in -WB/-JS and -WB/+JS than UN, +WB/-JS, and +WB/+JS ($p < 0.0001$; Fig. 5). Invasive richness of -WB/-JS had a minor increase compared with UN ($p = 0.0081$, Fig. 5). Native richness of -WB/-JS and -WB/+JS was nearly two fold greater than UN, +WB/-JS, and IN ($p < 0.0001$; Fig. 5). Plant community biodiversity in -WB/+JS was also greater than both IN and +WB/-JS ($p = 0.0241$; Fig. 5).

Organic matter, pH, phosphorous, potassium, calcium, magnesium, and zinc were not statistically different among the five treatments. Manganese was greater in UN than in all other treatments ($p = 0.0014$ and $p < 0.0001$ respectively), and copper was greater in UN than +WB/-JS and -WB/-JS but only in 2014 ($p = 0.0170$). Light penetration was highest in -WB/-JS, UN, and -WB/+JS, and lowest in +WB/+JS and +WB/-JS ($p < 0.0001$).

Cover-impact relationships:

The quadratic term for C_T and the linear term of C_{js}/C_T were significant for resident plant richness (Table 1, Fig. 6a); richness was lowest at high C_T and greatest (least suppressed) at 36%

cover. Resident richness was reduced linearly with C_{js}/C_T , and had 1.5 fewer species in high JS/low WB cover than in low JS/high WB cover (Table 1, Fig. 6a).

Invasive richness was strongly influenced by the quadratic C_T^2 (Table 1), and was 2 to 3-fold higher at moderate C_T than at low and high C_T . Also, the identity of the invader did not matter, only their combined cover (Fig. 6b). Native richness was reduced by nearly 70% with increasing C_T ; native richness peaked at <10% and was lowest at high C_T (Table 1, Fig. 6c). Also, JS more greatly reduced native richness than WB at any level of C_T (C_{js}/C_T $p=0.0355$). Therefore, relative to WB, JS had a greater effect on native than invasive species (Fig. 6c; Table 1). Community biodiversity was impacted by the interaction of C_T and C_{js}/C_T with JS more greatly reducing diversity at high C_T and WB more greatly reducing diversity at low C_T (Fig. 6d; Table 1).

Of species present in uninvaded plots only *Galium circaezans* ($p=0.0059$) and rare plants ($p=0.0361$) were less common with increasing C_{js}/C_T . Of species present in all plots, *Viola* spp ($p=0.0237$), *Galium circaezans* ($p=0.0059$), *Polystichum acrostichoides* ($p=0.0014$), and *Trillium grandiflorum* ($p=0.0163$) were less associated with increasing C_{js}/C_T . Therefore, ferns and forbs were also less present with increasing C_{js}/C_T . However, no species were more abundant with increasing C_{js}/C_T .

Soil properties were also significantly impacted by the presence of the invaders (Table 1). C_T had a linear effect on calcium, magnesium, boron, and copper; and a quadratic effect on copper and pH (Fig. 6e). C_{js}/C_T had a linear effect on iron and manganese, and a quadratic effect on phosphorus. Additionally, potassium and zinc were affected by the interaction of C_T and C_{js}/C_T .

Discussion:

Using a combination of observational and manipulative methods, we did not detect any synergies between JS and WB on the plant community—the invaders appear to have overlapping impact niches that vary only in magnitude but are non-synergistic. In the observational study, synergies would have been detected if the presence of both invaders (i.e., C_{js}/C_T) had a negative or positive non-linear change on plant richness. We found only linear relationships in all cases except for community biodiversity, but this interaction appeared to be weak and driven by small changes in native vs other invader species responses to JS. At similar total invader cover (C_T), JS had stronger reductions in resident plant richness than WB, and those reductions came primarily from native species. This was corroborated by our species presence analysis in the observational study: of the species present in uninvaded plots, only rare and one common native species were differentially impacted by these two dominant invaders. They were always less common in JS invasion. Similarly, of species present in the co-invasion, four native plants were less common with increasing JS invasion. There were no examples of any species being less common in WB invasion. This suggests that WB overlaps with JS in its impacts to the plant community, but JS appears to have a broader plant community impact niche which results in overall greater reductions in resident plant richness.

In the manipulative study synergies would have been indicated with the –WB/-JS plots having values either higher or lower than the single invader communities -WB/+JS and +WB/-JS, which would both be different than +WB/+JS. Instead, there was no change to the plant community whether JS was present with WB or not—WB was driving the reductions in richness. Furthermore, in the absence of WB, there was no difference in richness whether or not JS was present. However, this may be at least partially explained by the low intensity JS invasion (21%)

following WB removal (-WB/+JS) compared to heavy JS invasions in this region (80-100% JS cover). Cover of invasive plants can moderate their impact to the plant community as has been shown in grasses (Olsson et al. 2012), shrubs (Greene and Blossey 2011), trees (Ruwanza et al. 2013), and vines (Alvarez and Cushman 2002). In fact, we have previously found that the effect of JS on the resident plant community is strongly affected by JS cover—JS only began reducing richness at after a threshold of >60% cover (Tekiela and Barney 2015). Resident richness decreased by 40% from 20 to 80% JS cover (Tekiela and Barney 2015), which if we assume a similar relationship in the present study would put the -WB/+JS communities near the same lower richness as the communities with WB if JS had achieved a typical high cover. In any case, the present study demonstrates that at relatively low JS cover, WB is driving plant community dynamics.

JS and WB both appear to have overlapping impact niches in the forest understory, at least for plant and most soil impacts that do; however, vary in their magnitudes. Both species are small statured grasses that grow to similar heights, have similar morphology and phenology, and exist in similar habitats. Being ecologically similar, these species may have similar overlapping ecological impacts that are primarily linearly proportional to their cover. Although the magnitude of the impact was species specific in the observational study (i.e., JS more greatly reduced plant richness at equitable cover), JS and WB do not interact in a way to create impact synergies. This same conclusion was drawn in multi-invaded systems in the Western U.S. (Pearson et al. 2015). It should be noted that while we did record a variety of plant and soil parameters, which resulted in similar impact niches, this is by no means exhaustive as to the potential impacts caused by these species. Thus, their impact niches may indeed be different if additional parameters had been considered.

Conversely, species that do not share life histories or ecological niches may be more likely to have non-overlapping ecological impact niches that can result in positive or negative impact synergies. For example, Cushman and Gaffney (2010) showed that giant reed (*Arundo donax*), which is a large statured grass, and periwinkle (*Vinca minor*), which is a small vining herbaceous species, individually reduced native richness in riparian habitats, but in combination native plant richness was more greatly reduced (i.e., synergy). Synergies likely happen when the underlying mechanisms of impact are different. For example, even if two invaders show an equal impact on pH, the underlying mechanism could be different. One invader may induce changes to the microbial community composition while the other invader changes leaf litter production. This could easily be accounted for within the impact niche framework by also measuring these other impact metrics. Determining what metrics need to be studied for any species is difficult and will need to be further studied. If we were to perform this same study on two species such as Japanese stiltgrass and barberry (*Berberis thunbergii*) or Autumn olive (*Eleagnus umbellata*), we would likely identify synergies due to different underlying mechanisms of impact.

Interestingly, total invader cover (C_T) had a positive non-linear relationship with total resident plant richness, other invasive plant richness, and native plant richness; all values were highest at moderate C_T and lowest at low and/or high C_T . This surprising trend could suggest some level of facilitation or simply lack of competition for resources at moderate cover of JS and WB, and suppression, likely through competition at high C_T . A similar relationship has previously been shown in rugosa rose (*Rosa rugosa*), which had greatest species richness at approximately 20% cover (Thiele et al. 2011), *Hippophae rhamnoides*, which had greatest richness at 40% (Isermann 2007), and lantana (*Lantana camara L.*), which had greatest richness

at 30% (Gooden et al. 2009). Conversely, Pearson et al. (2015) looked at multi-invaded systems in the western U.S. and only found negative linear relationships with increasing invader cover. Nearly all other studies looking at invader cover only find negative linear relationships (Tekiela and Barney, unpublished data).

One possible explanation of this relationship is the intermediate disturbance hypothesis (Collins et al. 1995), which predicts the highest species diversity is in systems that experience moderate disturbance. The presence of invasive species have themselves been considered an ecological disturbance due to their impacts (Didham et al. 2005) and, therefore, increase plant richness. However, when present in high abundance, direct competition of the invasive plant locally excludes species. Additionally, at moderate total invader cover, pH was also highest (less acidic), which may have been more conducive to native and other invasive recruitment. Additionally, moderate cover of JS may have offered beneficial shade, which protected photosensitive understory plant from over-exposure (Krause et al. 1996).

Although no co-invasion-cover synergies were found on the plant community, both JG and WB impose negative impacts on the surrounding plant community whether co-invading or individually. Regardless of individual impacts of each plant, removal of both species did influence the plant community by increasing native plant richness and total biodiversity, though invasive plant richness was unchanged. Thus, management of these invaders provides immediate beneficial results.

In systems such as this, where the co-invasion is composed of species with overlapping impact niches, species identity may not matter, but total invader cover does. The widespread invasion of Japanese stiltgrass is already imposing strong impacts on the plant community. As wavyleaf basketgrass expands its range in the US, the resident plant community will not be

further impacted when co-invading with an existing heavy Japanese stiltgrass invasion. Thus, management and conservation goals should be aimed at reducing total invader cover, and not focus on individual species. This is especially promising in this case of two small understory grasses as it would not require species-specific management techniques, which may not be the case for dissimilar invaders (e.g., grass and shrub). Despite the similarity in impact niches for these invaders, they may differ in important ways, such as spread rates, that may influence management prioritization. Additionally, this study highlights the importance of incorporating invader cover in ecological impact studies. However, much remains to be determined in how invaders interact in co-invasions of two or more invaders, which better reflects the co-invaded nature of most ecosystems.

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Table 1. Multiple regression statistical t-test F-statistic and significance results for plant and soil metrics of observational study.

	C_T	C_{js}/C_T	C_T^2	$(C_{js}/C_T)^2$	$C_T * C_{js}/C_T$
Total resident richness	-5.61***	-2.28*	-5.21***		
Resident plant diversity			-2.00*		-2.20*
Native richness	-5.97***	-2.12*	-2.95**		
Invasive richness			-5.23***		
Organic Matter					
pH			-2.23*		
P				2.72**	
K					2.17*
B	2.65**				
Ca	3.44**				
Cu	3.72**		2.15*		
Fe		3.42**			
Mn		-2.08*			
Mg	2.67**				
Zn			2.61**		-2.11*

^a * signifies p-values <0.05, ** signifies p-values <0.01, *** signifies p values <0.0001

^b C_T is total cover, C_{js}/C_T is proportional cover

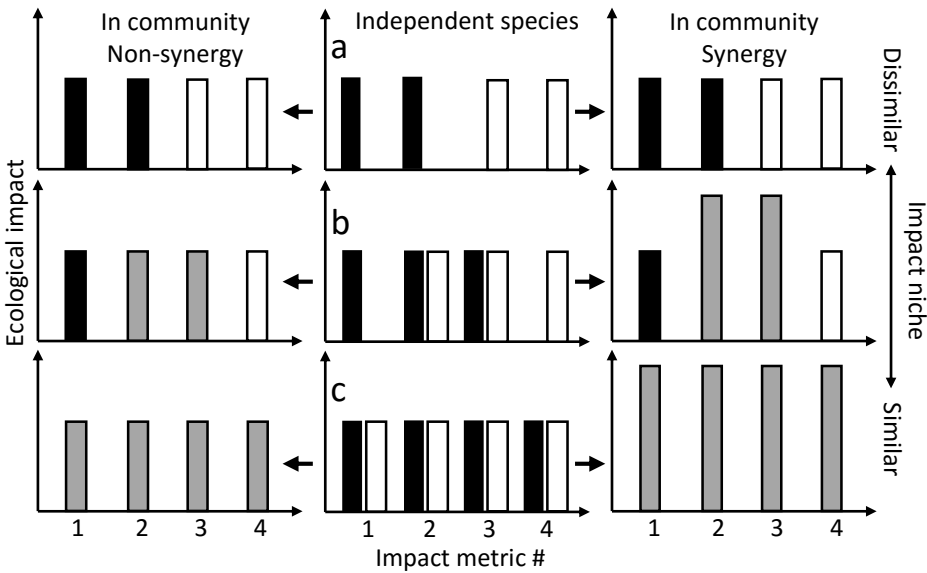


Fig. 1. Two invasive plant species represented by black and white bars that exist independently (center column) or in community (exterior columns). Each 'impact metric' is one of infinite measurable ecological impacts. Species with completely dissimilar non-overlapping impact niches (row a) cannot have synergies in community. Species with identical overlapping impact niches (row c) will interact at each impact metric (grey bars) but may result in either non-synergistic impacts where the combined impact is the average of the independent species (left column) or synergistic impacts where the impact is greater than the average of the independent species (right column) for each impact metric. Partial impact niche overlap can also occur (row b).

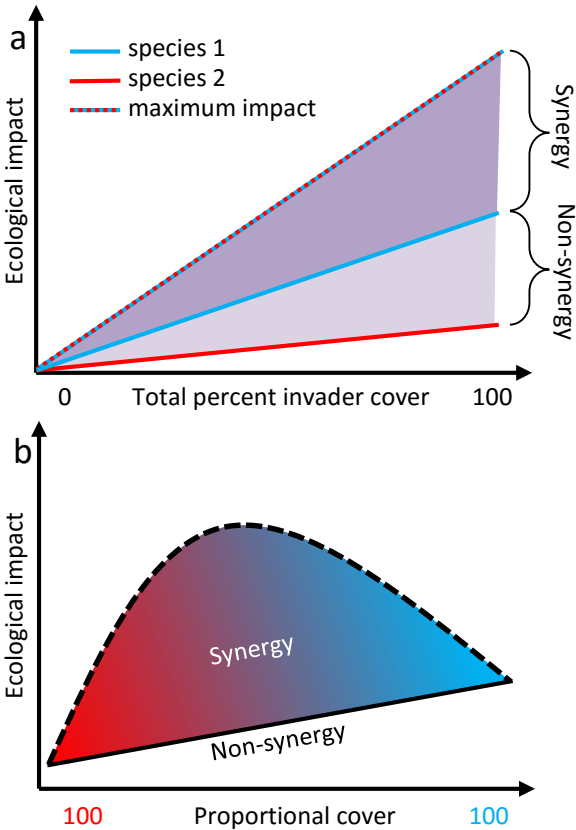


Fig. 2. Two invasive plant species' impacts are dependent on cover (panel a, blue and red line). When in community at any proportional cover (e.g., 50/50, 80/20) species are in non-synergy when the combined impact is the average of the two invader impacts (panel a, light-purple area) and in synergy when the combined impact is greater than the average of the two invader impacts (dark purple area). The maximum impact at any cover level is the sum of red and blue independent impacts (dashed blue-red line). Proportional cover of each species (panel b) shows a linear relationship when in non-synergy and a non-linear relationship when in synergy.

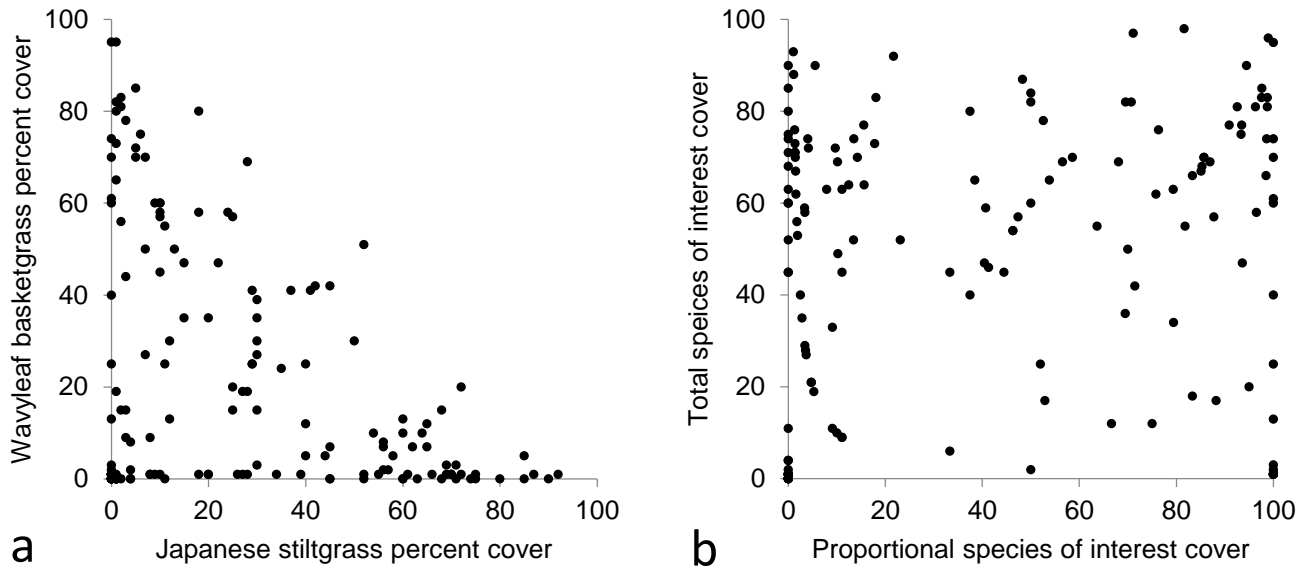


Fig. 3. Distribution of observational plots included in the observational study showing their total cover (C_T) and proportional cover (C_{js}/C_T) of Japanese stiltgrass and wavyleaf basketgrass on both untransformed axes (A) and transformed axes (B).

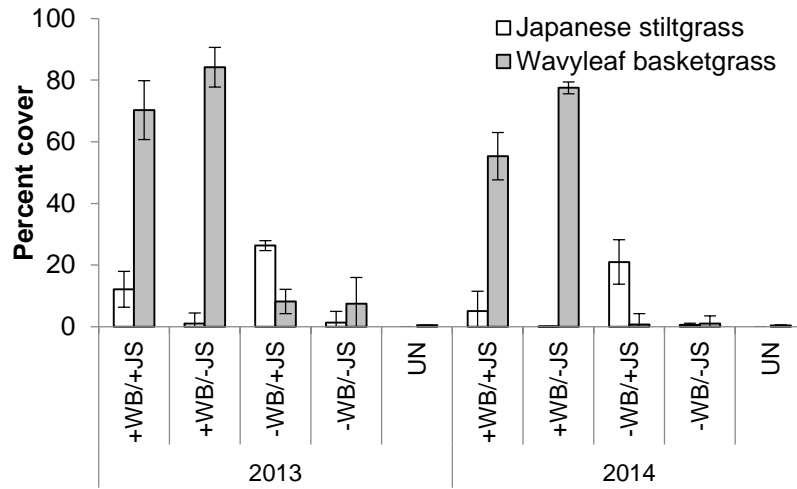


Fig. 4. Percent cover of invasive species of interest after removal treatments for 2013 and 2014.

+WB/+JS is invaded, +WB/-JS is selective Japanese stiltgrass removal, -WB/+JS is selective wavyleaf basketgrass removal, -WB/-JS is both removed, and UN is uninvaded reference site.

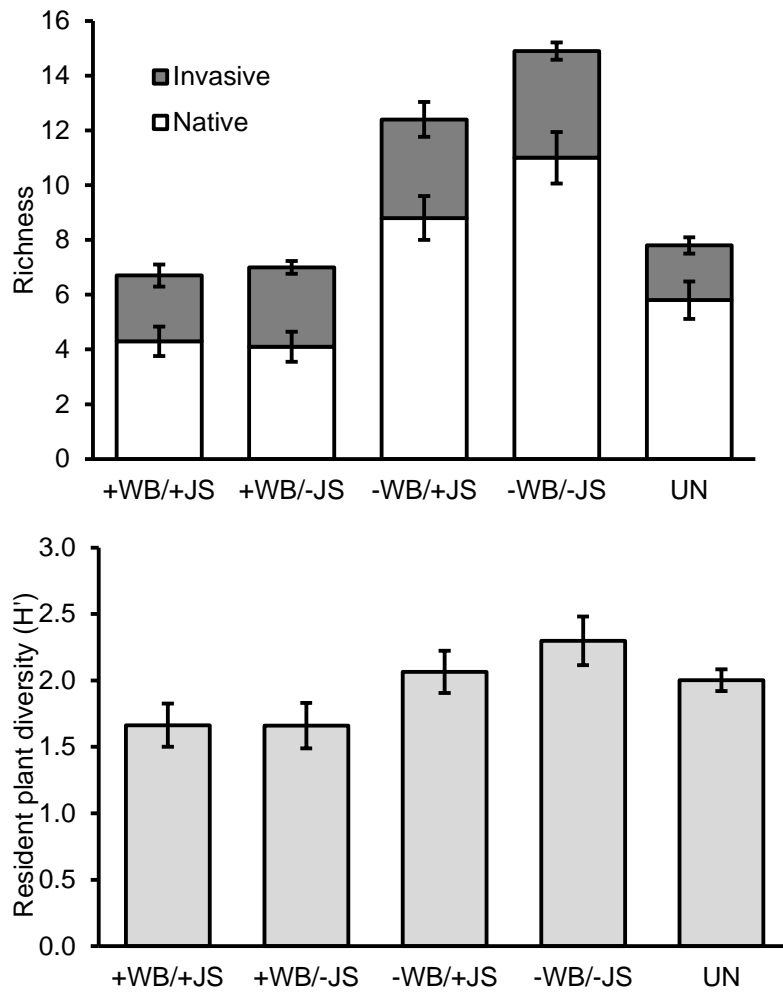


Fig. 5. Plant richness of native and other invasive species and biodiversity of resident plant community in manipulative study in 2014. For abbreviations see Fig. 3.

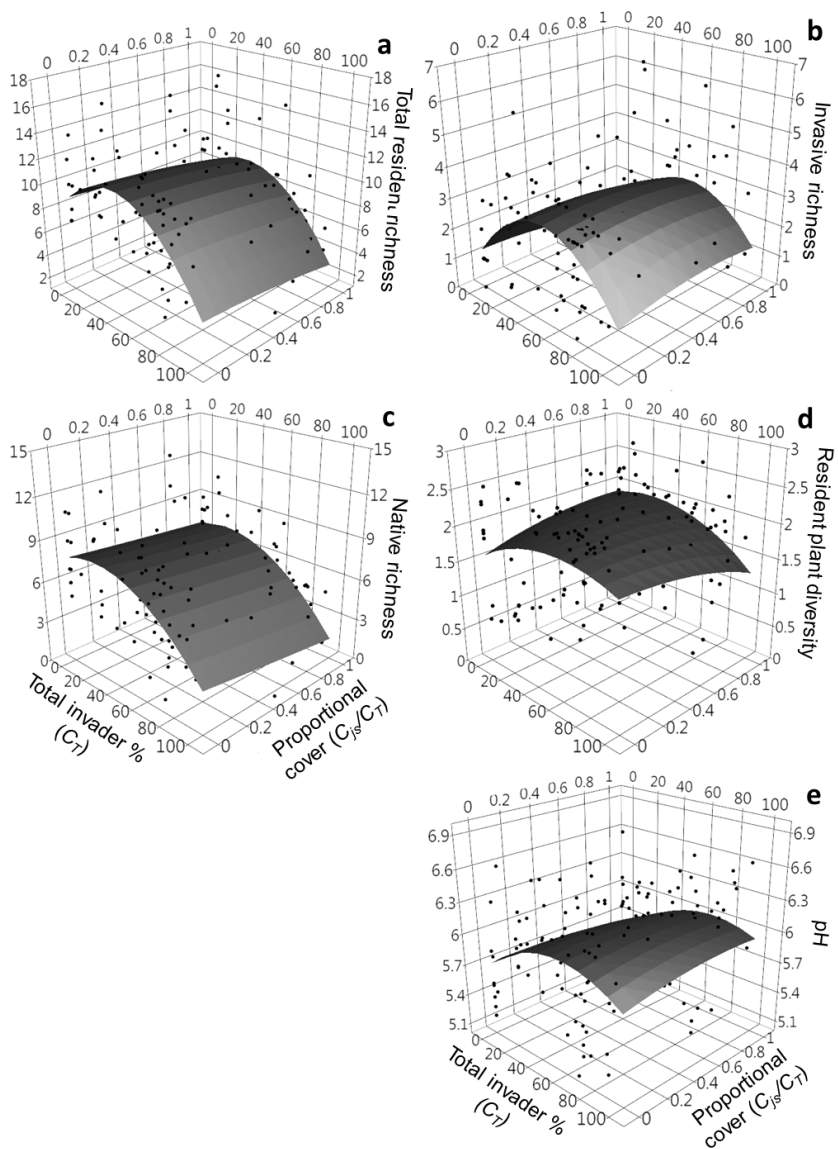


Fig. 6. Multiple regressions of total invader cover (C_T), proportional invader cover ($C_{j\#}/C_T$) and the responses of total resident richness (A), resident plant diversity (B), native richness (C), invasive richness (D), and pH (E).