Leaf Traits, Neighbors, and Abiotic factors: Ways That Context Can Mediate the Impact of Invasive Species on Nitrogen Cycling

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the University Program in Ecology
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ABSTRACT

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Abstract

Species invasions are more prevalent than ever before. While the addition of a species can dramatically change critical ecosystem processes, factors that mediate the direction and magnitude of those impacts have received less attention. A better understanding of the factors that mediate invasion impacts on ecosystem functioning is needed in order to target which exotic species will be most harmful and which systems are most vulnerable. The role of invasion on nitrogen (N) cycling is particularly important since N cycling controls ecosystem services that provision human health, e.g. plant productivity, ecosystem nutrient retention and water quality.

We conducted a meta-analysis and in-depth studies focused on the invasive grass species, *Microstegium vimineum*, to better understand how (i) plant characteristics, (ii) invader abundance and neighbor identity, and (iii) environmental conditions mediate the impacts of invasion on N pools and fluxes. The global meta-analysis confirmed and expanded upon prior synthesis efforts, showing a consistent positive effect of invasive species on N cycling and, for the first time, that dissimilarity in leaf and litter traits among invaded and reference plant communities control the magnitude and direction of invasion impacts on N cycling. Regarding the in-depth studies of *Microstegium*, we did not find that it increases nitrate or net nitrification as other studies

have shown. In the greenhouse, more plant biomass, regardless of species identity, drove decreases in soil moisture and inorganic N concentrations, whereas interactions among neighboring species mediated net N mineralization rates. In field surveys, we found that more *Microstegium* biomass led to increases in soil nitrate in areas with high light and few trees, whereas the opposite trend was observed at sites with greater forest openness. Invasion also promoted net ammonification rates through direct and indirect pathways such that greater increases in net ammonification were generally observed either (a) where there is low soil moisture and organic matter, or (b) where there is high soil moisture and organic matter and (i) high *Microstegium* biomass or (ii) low forest openness. Collectively, our findings suggest that dissimilarity in plant community traits, neighbor identity, and environmental conditions can be important drivers of invasion impacts on ecosystem N cycling and should be considered when evaluating the ecosystem impacts of invasive species across heterogeneous landscapes.

Dedication

This dissertation is dedicated to my parents and grandparents. Thank you, Martha and Jim Lee, Ruth and August Meyers, and Emily and Carleton Lee, for your constant love and support.

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1. Introduction

Globally, rates of species invasions and extinctions have moved well beyond known historical values (Steffen *et al.* 2015). In addition, there is accumulating evidence that species' additions and losses can dramatically alter ecosystem processes that provision human and ecosystem health. But which species have large ecosystem effects and in what contexts? One of the grand challenges in ecology today is to address this question and develop the ability to predict and mediate the consequences of shrinking and shifting biodiversity across the globe.

A better understanding of how individual species contribute to an ecosystem process can be gained by studying plant invasions (Ehrenfeld 2003). Plant invasions serve as a type of "natural experiment" since they involve the addition of a species to a naïve system. Thanks to the work of many researchers over the past 20 to 30 years as well as recent synthesis efforts, we know that plant invasions can severely impair ecosystem services, such as nutrient retention, water quality, carbon storage, and the reduction of greenhouse gas emissions (Liao *et al.* 2008; Vilà *et al.* 2011). While invasions can impair ecosystem services and be quite costly, not all invasive species or invaded systems experience invasion impacts to the same degree, or even in the same direction (Castro-Díez *et al.* 2014). As such, we must learn to predict which species are most harmful and which ecosystems are most vulnerable in order to effectively allocate limited resources to manage the consequences of invasion (Hulme *et al.* 2013). Moreover, there is a clear need to better understand factors that mediate the impact of invasion on ecosystem processes, since invasion impacts are often difficult to detect early in the invasion process and expensive and intractable to remediate for established invaders (Strayer 2012; Funk *et al.* 2014; Gaertner *et al.* 2014).

The goal of this dissertation is to better understand how characteristics of invasive plant species and naïve plant communities and ecosystems shape the nature of invasion impacts on soil nitrogen cycling. Nitrogen (N) is a key element that is critical to plant growth and ecosystem development (Schlesinger & Bernhardt 2013). In addition, soil N pools and fluxes are linked to a number of ecosystem processes that provision ecosystem and human health, i.e. nutrient retention, water quality, and the reduction of greenhouse gas emissions (Ehrenfeld 2003).

To gain a better understanding of factors that mediate invader impacts on soil N cycling, I took two distinct yet complementary approaches. The meta-analytic (Chapter 2) and model-system (Chapters 3 & 4) approaches complement one another in addressing the primary motivation of the dissertation. Together they convey the breadth and depth of our current understanding of factors that mediate invasion impacts on soil N pools and fluxes.

In Chapter 2, I present the results of a meta-analysis that looks across invasive species and invaded systems, globally, to evaluate whether invasive species and invaded community characteristics explain variation in invasion impacts on soil N cycling measures. This synthesis is the most comprehensive analysis of invasive plant impacts on soil N cycling to date, including 143 published articles on the topic. There is a growing consensus that plant invasions increase soil N pools and fluxes, but invasion can impact soil N cycling through a variety of mechanisms (Knops, Bradley & Wedin 2002; Laungani & Knops 2009). This chapter answers the outstanding question of whether plant traits like leaf and litter chemistry contribute to variation in invasive impacts. In addition to investigating traits of the invasive species, we investigated reference plant community traits, and dissimilarity among invader and reference traits, and the role of study design, as potential drivers of variation in invasive species impacts on N cycling. Some invasive species' traits, reference community traits, and trait dissimilarities explained aspects of invader impacts on N cycling, including invasive species' leaf and litter %N, reference community leaf C:N, and dissimilarities in litter %N and leaf and litter C:N. We did not detect associations that contradicted our hypotheses, but many of the plant characteristics that we expected to explain variation in soil N effect sizes did not. Overall, the most informative traits were the trait dissimilarities, not invader trait values. We found that study design had relatively minor effects on impact magnitudes. Our results support the concept that traits are useful for understanding species' effects on ecosystem function but suggest that trait dissimilarity is critical for understanding invader impacts on N cycling.

In Chapters 3 & 4, I present the results from studies that focus on understanding factors that mediate the impact of one invasive species, *Microstegium vimineum*. This invasive grass is one of the most highly studied invasive plant species, especially with regard to its impact on soil N pools and fluxes (Castro-Díez *et al.* 2014), and so can serve as a model system for in-depth study of factors that mediate variability in invader impacts on N cycling.

The greenhouse study presented in Chapter 3 uses *Microstegium* to address (i) whether a species' effect on an ecosystem process scales with its abundance and (ii) whether this relationship between invader abundance and ecosystem process is mediated by the identity of neighboring plant species. It is generally assumed that a species' effect on an ecosystem process scales with its abundance, but this is rarely tested. In addition, neighboring plant species may be important in mediating the magnitude and direction of a species' effect by influencing pathways through which the target species alters an ecosystem process. Previous studies have found that *Microstegium* increases N mineralization, particularly nitrification rates where it invades (Kourtev, Huang & Ehrenfeld 1999; Ehrenfeld, Kourtev & Huang 2001; Kourtev, Ehrenfeld & Haggblom 2002; Lee, Flory & Phillips 2012), so we tested how the effects of *Microstegium* N mineralization rates scaled with its abundance, with and without neighbors of different identities. *Microstegium* was grown in the greenhouse in the absence and presence of either *Panicum virgatum*, which naturally co-occurs with

Microstegium in its invasive range, or *Sorghum bicolor*, which produces nitrification-inhibiting compounds. We did not find evidence that the invader increases nitrification when grown alone or with neighbors, but the interaction between *Microstegium* biomass and neighbor treatment were important for determining nitrate concentrations and net nitrification rates. As such, the results of our study serve as a cautionary tale against using solely a linear factor of invader density to estimate its impact on soil properties, particularly across areas where neighbor identity varies.

The field study presented in Chapter 4 uses naturally-occurring *Microstegium* invasions in the southern piedmont to address how environmental conditions can mediate the magnitude and direction of invasion impacts on N cycling directly and indirectly by controlling invader biomass. We determined the relative importance of these direct and indirect pathways using a structural equation modeling approach applied to data from a network of invasion front sites varying in invader biomass, soil organic matter, soil moisture, and light availability. We found that forest openness promotes the invader's biomass, but we did not find evidence that the invader's biomass promotes its impact on soil N pools or fluxes, except in the case of ammonification. Site-level impacts of invasion on nitrate pools were mediated by a negative interaction among forest openness and invader biomass such that no overall effects of invasion on nitrate or nitrification were detected across sites. Overall, invaded plots had higher net ammonification than reference plots, but in this case too, site-level interactions and indirect effects among *Microstegium* biomass and environmental conditions were observed. Notably, we detected a scenario in which forest openness has a negative direct effect *and* indirect positive effect on ammonification in sites with high soil moisture and organic matter. A better understanding of how environmental conditions and invader biomass interactively and indirectly mediate invasion impacts will help land managers target areas that are likely to experience large invasion impacts. Moreover, these findings emphasize the need to incorporate interactive and indirect effects of environmental context into our estimates of species effects on soil processes.

Chapter 5 integrates the conclusions from the previous chapters. This includes a discussion of using functional traits and the difference in community-weighted mean trait values to understand species' effects on soil N pools and fluxes. Sometimes, as we found in the greenhouse study on *Microstegium*'s impacts on soil N cycling, the strong actors are the neighboring species instead of the focal invasive species. Chapter 5 also discusses our finding that environmental factors can simultaneously mediate invasion impacts directly, indirectly and interactively and that these multiple pathways can lead to unexpected situations where same environmental factor that promotes invader abundance can also limit its impact on an ecosystem process at a given site. From this research, we better understand how, across the globe and for a well-studied model invasive species, characteristics of an invasive species, the invaded plant community, and the invaded ecosystem shape the consequences of invasions for soil N cycling.

2. Invasive species' leaf and litter traits, but especially trait novelty, shape their impact on nitrogen cycling

2.1 Introduction

Globalization has increased rates of invasion by non-native species (Sax, Stachowicz & Gaines 2005). While some exotic species have little apparent impact on ecosystem processes, some exotic species have dramatic consequences for human and ecosystem health, impairing ecosystem services, harming native species, and promoting further invasions (Ehrenfeld 2003; Steffen *et al.* 2015). There is a growing consensus that plant invasions increase soil nitrogen (N) pools and fluxes, speeding-up N cycling in ways that can be harmful for people and ecosystems by reducing ecosystem nutrient retention, water quality, and carbon storage, and increasing greenhouse gas emissions (Ehrenfeld 2003; Hickman & Lerdau 2013). A number of global synthesis studies have demonstrated that plant invasions are associated with faster N fluxes and larger N pools (Ehrenfeld 2003; Liao *et al.* 2008; Vilà *et al.* 2011; Pyšek *et al.* 2012). Liao *et al.* (2008) found that, on average, invaded areas have 50% faster soil N mineralization and nitrification fluxes and 30% and 17% greater soil ammonium and nitrate pools, respectively, than their non-invaded counterparts. But, while these biogeochemical changes can be large, there is significant variability in the direction and magnitude of invasion' impacts that remain unexplained. We need a better understanding of factors that mediate the impacts on invasion in order to anticipate and remediate the consequences (Pyšek *et al.* 2012; Hulme *et al.* 2013). After a brief summary of previous syntheses, we pick up where others have left off and evaluate whether leaf and litter plant functional traits, specifically invasive species' traits, reference community traits, and community trait dissimilarities, improve our understanding of the drivers and mediators of invasive impacts on N cycling.

Invasive species can impact soil N cycling through a variety of mechanisms. For example, they can alter the activity or composition of microbial N-transformers and the quality or quantity of plant N uptake and release (Knops *et al.* 2002; Hawkes *et al.* 2005; Chapman *et al.* 2006). In addition to characteristics of invasive species themselves, there are a number of factors that can mediate the impacts on invasion on soil N cycling, including characteristics of resident species and environmental conditions. A few synthesis studies have begun to investigate the role of plant characteristics and climate conditions in mediating the impacts of invasion on soil processes (Liao *et al.* 2008; Vilà *et al.* 2011; Castro-Díez *et al.* 2014), but much work still remains. The mediating factor that has received most attention, with good reason, has been whether the invasive species associates with N-fixing bacteria (hereafter, "N-fixing species"). On average, N-fixing invasive species have larger soil N effect sizes than non-N-fixing invasive species (Liao *et al.* 2008; Vilà *et al.* 2011; Castro-Díez *et al.* 2014), and this is especially true in cases where the invaded community lacks N-fixers (Castro-Díez *et al.* 2014). However, even non-N-fixing invasive species are associated with increases in soil N pools and fluxes (Liao *et al.* 2008; Castro-Díez *et al.* 2014) and it remains unknown whether non-N-fixing invasive species tend to increase soil N pools and fluxes in cases when they invade areas with resident N-fixing species.

Although much of the focus has been on species' N-fixing status, plants can control soil nutrient processes by differing in the ways they take up and release nutrients (Wedin & Tilman 1990; Chapman *et al.* 2006). With different nutrient-use strategies, plant species can mediate soil N pools and fluxes by having either fast nutrient uptake and release, promoting fast N cycling, or slow nutrient uptake and release, promoting slow N cycling (Knops *et al.* 2002; Chapman *et al.* 2006; Laungani & Knops 2009). Plant functional traits, such as leaf N concentration and carbon-to-nitrogen (C:N) ratio, are a common currency that can be used to characterize how species effect ecosystem processes (Suding, Lavorel & Chapin 2008; Reich 2014). Specifically, nutrient concentrations and ratios in plant species' leaf and litter tissue can provide information about how plant species vary in their nutrient-use strategy and thus vary in their impacts on soil N cycling. There is evidence, across the globe, that invasive species have leaf traits that indicate that they have faster nutrient-use strategies than native species (van Kleunen, Weber & Fischer 2010; Ordonez, Wright & Olff 2010). Do these traits drive invasion impacts on N cycling? Evidence from case studies suggests that this is an important factor driving invasive species' impacts on N cycling, especially for non-N-fixing invasive species (Godoy *et al.* 2009; González-Muñoz, Castro-Díez & Parker 2013). On a global scale, however, the role of invasive species' leaf and litter traits in driving the direction and magnitude of invasion impacts on soil N cycling has not been considered in previous meta-analysis reviews

The plant community in which the invasion event takes place can have an important influence on the speed, magnitude, and/or direction on N cycling changes following invasion. In other words, characteristics of the community that exist prior to the invasion event, or the reference community, can mediate invasion impacts on N cycling. A previous synthesis supports this general hypothesis, finding that invasive N-fixing plants cause stronger impacts on N pools in communities lacking N-fixers (Castro-Díez *et al.* 2014). In addition to the N-fixing status of reference communities, community-weighted mean (CWM) tissue trait values from the reference community are likely to be important since they reflect the cumulative effect of plant species on soil N processes, weighted by species' relative abundance. The CWM trait values of a reference community can also, by extension, provide information about N cycling pool sizes and flux rates in reference area soils (Cornwell *et al.* 2008; Craine *et al.* 2009), and these baseline levels may be extreme enough that increases or decreases are impossible because of biogeochemical constraints. For example, the increases in N cycling are less likely for systems with extremely N-rich reference communities since invasive species' traits cannot be more N-rich. As of yet, the roles of reference CWM leaf and litter traits in mediating invasion impacts on soil N cycling are untested.

The relative difference between invasive species and native species trait values can predict the impact of invasion on N cycling because it represents the shift in the plant community functional traits that shape soil N pools and fluxes (Godoy *et al.* 2009; González-Muñoz *et al.* 2013; Castro-Díez *et al.* 2014). Ecosystem impacts, however, are measured and calculated by sampling invaded and reference areas. Invaded areas amount to more than simply the traits of the focal invasive species and, similarly, reference areas often include non-native species. As such, dissimilarity between CWM trait values of the invaded and reference area may be a more informative dissimilarity measure, since it more closely matches how ecosystem impacts are measured and calculated. A previous meta-analysis reported that litter material in invaded areas has, on average, a 30% lower C:N ratio than reference plots (Liao *et al.* 2008), which begs the question – does dissimilarity in leaf and litter plant traits among the invaded and reference communities drive the direction and magnitude of N cycling responses observed? Invasive species can impact N cycling directly via their nutrient-use traits and indirectly via displacing native plant species that are likewise important in shaping N pools and fluxes. Trait dissimilarity addresses both of those impact pathways. In addition, a third mechanism of trait dissimilarity effects is that new combinations of trait values may have synergistic impacts on N cycling. Whether the addition of litter that is much higher in quality promotes larger than expected or smaller than expected impacts on net N mineralization due to microbial community mediation is the subject of continued debate in the litter decomposition literature (Strickland *et al.* 2009; Pearse, Cobb & Karban 2013). For example, there is evidence that litter mixtures with a diversity of tissue qualities (e.g. high and low litter %N values) undergo decomposition faster than homogenous mixtures due to a complementary suite of resources for decomposing organisms (Gartner & Cardon 2004). However, other studies find that decomposition is more rapid for litter mixtures that most closely match the litter quality to which the decomposing organisms are accustomed (e.g. high or low litter %N), exhibiting a 'home-field advantage' (Smith & Bradford 2003; Strickland *et al.* 2009).

Finally, study design may be an important factor in mediating the magnitude and direction of measured invasion impacts. All approaches have limitations. Observational studies cannot isolate causality (Davis *et al.* 2011) and are often measuring the effects of long term invasion. Experimental results are contingent on whether invaders are added (Lee *et al.* 2012) or removed (DeMeester & deB Richter 2010). Greenhouse studies are constrained by pot sizes, duration, and lack of community effects such as litter accumulation (Lee *et al.* 2012). A previous meta-analysis conduced by Castro-Díez *et al.* (2014) found no difference in invasion effect sizes on soil N pools and fluxes based on whether the study was observational, experimental, or a combination, which suggests that invasion impacts are in fact a consequence of invasion. It is important to continue to monitor this potential signal as we add new studies to our analyses.

Our objectives in this meta-analysis are three-fold. First, we update the previous meta-analyses on N cycling responses to invasion (Liao *et al.* 2008; Vilà *et al.* 2011; Castro-Díez *et al.* 2014) with 67 newly added publications which constitutes 47% of the full dataset. With this more robust dataset, we then assess whether the magnitude and direction of N cycling responses to invasion are a function of invasive species characteristics, reference community characteristics, and their dissimilarities. We expect greater impacts where the invasive species is N-fixing and has N-rich trait values (e.g. high %N and low C:N). We expect more modest N cycling shifts in systems where N-fixing species are present in the reference community and where the reference community has N-rich CWM trait values. We expect larger impacts where trait dissimilarity is large among invaded and reference communities and predict that trait dissimilarities will best predict the direction and magnitude of invasion impacts. Third, we identify the role of study type on variation in effect sizes, expecting greater impacts in observational than experimental studies due to environmental filtering and potentially longer time-since-invasion, and greater impacts in experimental field addition than removal studies due to lag-effects. Addressing these aims will clarify how, on a global scale, plant invasions impact soil N cycling and which factors mediate the direction and magnitude of those impacts.

2.2 Methods

2.2.1 Study selection

The aim of the literature search was to collect observational and experimental studies that measure soil nitrogen properties in invaded and reference areas. We evaluated papers cited in Liao *et al.* 2008 and conducted database searches to collect articles published after 2007 and any articles published prior to 2007 that may have been overlooked. The search terms for the two Web of Science queries that were conducted on 11/17/14 were (1) (("invasion" OR "invasive") AND ("nitrogen cycling" OR "soil nitrogen") AND ("plant" OR "grass")) and (2) (("invasion" OR "invasive") AND ("soil nitrogen" OR "nitrogen cycling" OR "soil nutrient")). Related records were also identified if they were cited within any of the full-text articles assessed for eligibility, but were not captured in the literature search. Of the 483 unique articles that were identified, a total of 143 articles were acceptable for inclusion in this meta-analysis (see Supplement: Figure 3 for a flow chart of the article identification and selection process and Supplement: Table 4). A total of 67 articles (46.9%) are unique to this meta-analysis dataset (Supplement: Table 4). The following criteria were used to identify acceptable articles: (1) article must present data on the relationship between an invasive plant species or set of invasive species (up to 5 species) and soil nitrogen measurements, (2) measurements must be taken in both an invaded and a reference area, (3) must include at least one of a the following measurement types: inorganic N pools, ammonification, nitrification, mineralization, or total soil N, and (4) measurements must reflect a space-for-time substitution.

2.2.2 Study-level measurements and effect size calculation

Data were collected within accepted articles from tables, as available, or from graphs using ImageJ (Rasband 1997). Articles often included the results of multiple invaded and reference area comparisons. For example, some articles evaluated multiple invaded areas, targeting different focal invasive species, and compared those findings to one type of reference area. As in previous meta-analyses, we extracted all possible invaded and reference area comparisons reported within each article (van Kleunen *et al.* 2010; Vilà *et al.* 2011; Castro-Díez *et al.* 2014; Leffler *et al.* 2014). Each unique comparison we termed a "study". Most articles included more than one study, so we accounted for this nested quality of the data structure in our meta-analysis models. Studies within the same article were considered distinct if they presented data from different study types (e.g. field observation and greenhouse study) or sites. Data from repeated measures were aggregated as in Liao *et al* (2008).

As available, the following soil measurement data from within invaded and reference plots were collected from each study: soil inorganic N pool concentrations (ammonium, nitrate, total inorganic N), net or gross inorganic N mineralization rates (ammonification, nitrification, mineralization), soil organic matter, total soil N, and soil C:N. CWM traits included leaf %N, litter %N, leaf C:N, and litter C:N for invaded and reference area plant communities (Supplement: Table 4). Invasion effect sizes were calculated using Hedges' d effect-size statistics (Hedges 1981; Viechtbauer 2010). This effect size metric was used in two of the previous meta-analyses on this topic (Vilà *et al.* 2011; Castro-Díez *et al.* 2014) and was chosen instead of the log response ratio used by Liao *et al.* (2008) because N mineralization measurements are often measured as net rates and thus can take on negative values. We calculated one effect size for each soil measurement collected from each study in such a way that the difference in the soil measurement value between the invaded and reference area was normalized by the pooled standard deviation and a sample-size weighting factor (Supplemental Text Equation 1). Effect sizes greater than zero indicate that measurement values are higher in invaded than reference areas. Study effect sizes were calculated using the *escalc* function with measure="SMD" in the *metafor* package of R (Viechtbauer 2010).

2.2.3 Community-weighted mean (CWM) trait estimates and dissimilarities

CWM trait values, calculated as the abundance-weighted mean of the traits of plant species in a community, were collected or estimated for four plant traits (leaf %N, litter %N, leaf C:N, and litter C:N) and three species pools (hereafter, "community types"): (1) the invasive species, (2) all plant species present in the invaded area, and (3) all plant species present in the reference area. Note that the first "community type," invasive species, is often a single species; however, some studies followed co-occurring invaders so we treated these species as a community for the purpose of calculating at representative mean trait value. CWM traits were only calculated in 14% (leaf %N), 5% (leaf C:N), 13% (litter %N), 12% (litter C:N) of studies (Supplement: Table 5). For studies that did not report CWM trait data in invaded or reference areas, we calculated CWM trait values for each community type by combining study-specific species' lists and relative abundances with trait data from the TRY database (Kattge *et al.* 2011). If species' percent cover were not reported, this data was estimated based on the author's description of the study design. For example, many authors define whether a plot is invaded based on a minimum level of invader cover, e.g. 80%. If the author provided no description of species' relative abundances in invaded and reference plots, then we chose to assume that the species listed without information were present in equal relative abundance. This assumption was necessary for 19% (leaf %N), 22% (leaf C:N), 45% (litter %N), 21% (litter C:N) of CWM trait values on average across community types (Supplement: Table 5). Trait data were assigned to species using the highest quality data available from among the following sources (high to low quality): (1) species' mean study-reported trait estimate, (2) species' mean TRY database trait estimate, and (3) genus' mean TRY database trait estimate (Supplement: Table 5). CWM trait values were only included in our analyses if the observation had at least one invasive and reference species, with each species having a mean trait value for the trait of interest and estimates for the species' abundances in the invaded area and reference area. CWM trait dissimilarity among invaded area and reference area CWM traits was calculated as the difference in CWM trait estimates in such a way that higher trait dissimilarity values indicate higher CWM trait values in the invaded than the reference plant community. We were able to calculate CWM leaf %N values for invasive species and reference communities, and the dissimilarity in invaded and reference CWM leaf %N for the majority of studies (90%, 79%, and 79%, respectively; Table 1), whereas it was more difficult to assign CWM values for the other trait types: leaf C:N, litter %N, and litter C:N (Table 1; see Supplement: Figure 4 for trait frequency distributions).

Table 1: Predictor variables used to explain variation in invasion impacts on soil N cycling in this study. For categorical variables, the number of studies (k) per level are shown and percent of studies (%k) per level sums to 100. Data availability restricted the coverage of trait-based data such that the percent of studies with trait data is less than 100%

Predictor		Level	Studies (k)	Studies (k/k_{total})*100%	New studies (k_{new}/k) x100%
Invasive species' traits	Leaf %N	*continuous*	364	90.1	54.1
	Litter %N	*continuous*	50	12.4	32.0
	Leaf C:N	*continuous*	208	51.5	50.0
	Litter C:N	*continuous*	47	11.6	53.2
Reference CWMs	Leaf %N	*continuous*	320	79.2	53.4
	Litter %N	*continuous*	59	14.6	33.9
	Leaf C:N	*continuous*	155	38.4	49.0
	Litter C:N	*continuous*	54	13.4	48.1
Trait dissimilarity (Inv.-Ref. CWM)	Leaf %N	*continuous*	318	78.7	53.8
	Litter %N	*continuous*	59	14.6	33.9
	Leaf C:N	*continuous*	155	38.4	49.0
	Litter C:N	*continuous*	54	13.4	48.1
N-fixing status		No N-fixers	295	73.0	58.6
		Ref. community N-fixers only	38	9.4	42.1
		Invasive sp. N-fixers only	51	12.6	43.1
		Ref. and inv. sp. N-fixers	20	5.0	70.0
Study design		Field observation	273	67.6	43.2
		Field addition	45	11.1	73.3
		Field removal	25	6.2	68.0
		Greenhouse study	61	15.1	93.4

A consequence of patching together data sources to calculate CWM trait values is that these values vary in quality. To qualitatively evaluate the significance of CWM data quality in our analyses, we computed a quality rank for each CWM value from zero to four such that values with the highest quality have high quality rank values. All CWM values reported in the original study have a quality rank of four. Calculated CWM values can be a maximum quality rank of three and were awarded one point based on whether they met any of the following criteria: more than 25% of species within the community have (i) cover measurements from within the original study (either species-specific or -aggregated), (ii) species-specific cover, and (iii) species-specific trait value (Supplement: Table 5). The significance on these criteria are that CWM values are low quality if they are computed with species' cover estimates (i) based on the author's description, (ii) based on the assumption that species' are equally abundant, or (iii) using genus-level TRY trait data. CWM trait dissimilarities are also assigned a quality rank, calculated as simply the sum of the invaded area and reference area CWM quality ranks from which it was derived (i.e. maximum quality rank = 8).

We were also interested in the role of N-fixing species' presence in invaded and reference areas (hereafter 'N-fixing status) and study design as factors that mediate the impact of invasive plants on soil N cycling. Therefore, we categorized studies into an N-fixing status and study type (Table 1). N-fixing species were identified using the study-specific species lists and a list of *Frankia*-associated and Actinorhizal plant genera from The Plant List (accessed May 11, 2015, http://www.theplantlist.org/).

2.2.4 Statistical analyses

We estimated a grand effect size for each soil measurement type in our analyses using linear mixed models, performed with the *rma.mv* function and the REML (restricted maximum likelihood) method in the metafor package in R version 3.2.3 (Viechtbauer 2010). Each model included nested random effects of study within paper to account for the structure of the dataset. Study effect sizes were evaluated for normality to address model assumptions. In the context of this model, the estimated intercept parameter represents the estimated grand effect size. A test for residual heterogeneity was carried out using the X^2 distribution to determine whether variability in the observed effect sizes or outcomes is larger than one would expect based on sampling variability. Parameter estimates, 95% confidence intervals, and variance components were also obtained from *rma.mv* output.

To evaluate the role of factors that may mediate those effect sizes, we created models to test the significance of the following fixed effects: (a) invasive species' leaf or litter trait values, (b) reference CWM trait values, (c) dissimilarity in CWM trait values, (d) N-fixing status, and (e) study design. We did not construct models with multiple fixed effects because of the abundance of missing data across studies. In order to identify informative effect size models based a fixed effect, we identified models with a significant fixed effect based on a test of residual heterogeneity. This tests whether the variability in the observed effect sizes or outcomes that is not accounted for by the moderators included in the model is larger than one would expect based on sampling variability. We evaluated the model fit by calculating a pseudo-R^2 value based on the proportion of variance explained in the full model relative to a reduced model without the fixed effect (Supplemental Text Equation 2). To evaluate the role of CWM data quality, we evaluated the importance of including quality rank as a fixed effect and visually inspected quality rank in scatterplots of significant trait by effect size relationships. For categorical fixed effects such as N-fixing status and study design, we ran post-hoc Wald-type tests to evaluate differences among factor levels.

Meta-analyses can be subject to publication bias (Koricheva, Gurevitch & Mengersen 2013). For each effect type, we evaluated publication bias in our dataset by testing for correlation among study effect sizes and samples sizes, looking for asymmetry in funnel plots, and calculating the fail-safe number (Koricheva *et al.* 2013; Castro-Díez *et al.* 2014). We did not detect a significant correlation among study effect sizes and sample sizes for all effect types except total inorganic N effect sizes, which suggests that larger effects sizes in one direction are not more likely to be published for most effect types (Supplement: Table 6). Larger total inorganic N effect sizes, however, may be more likely to be published, and this should be considered along with the results we present. Plots of effect size and standard error exhibit symmetrical to marginally skewed funnel-shaped distributions. Again, skew is an indication of publication bias that may influence nitrate, total inorganic, and soil N effect sizes based on inspection of funnel plots (Supplement: Figure 5). The fail-safe number is the number of studies that would need to be added to the dataset to make the grand effect size statistically non-significant. Using the Rothenthal method to calculate fail-safe numbers, we found larger than expected fail safe numbers for all of the significant grand effect sizes we detected (Rosenthal 1979), which suggests that our results are reliable estimates of the true effect.

All statistical analyses were conducted using R Statistical Software (R Development Core Team and Team 2012). The R packages plyr and ggplot2 were used to manipulate and visualize data (Wickham 2009, 2011).

2.3 Results

2.3.1 Global effect sizes

On average, invaded areas have significantly larger ammonium pools (Hedges' d ± 95%CI: 0.26±0.14), faster nitrification (0.45±0.24), faster mineralization rates (0.36±0.22), more total soil N (0.53±0.22), and more soil organic matter (0.58±0.40) than reference areas (Table 2, all $p<.05$). The global mean of total inorganic N effect sizes is also slightly positive but not significantly different from zero (Table 2). Ammonification rates and soil C:N values do not significantly differ in invaded relative to reference areas (Table 2).

Table 2: Invasion tends to promote larger and faster soil N pools and fluxes based on four meta-analyses, including this study. Invasion effect size values for 9 measurements that characterize soil inorganic N pools, fluxes, soil N, C:N and organic matter; mean +/- 95% confidence interval with the number of studies, k. All effect size values are calculated as Hedges' d (d^+), except Liao *et al.* 2008, which is calculated as a weighted response ratio (RR_{++}). Grey highlight indicates that the 95% confidence interval does not overlap zero. Effect sizes >0 indicate that measurement values are greater in invaded than reference areas

Soil measurement		This study			Liao *et al.* 2008			Vilà *et al.* 2011			Castro-Díez *et al.* 2014		
		Effect size (d^+)	95% CI	k	Effect size (RR_{++})	95% CI	k	Effect size (d^+)	95% CI	k	Effect size (d^+)	95% CI	k
Inorganic N pool	NH_4^+	0.26	0.12 to 0.40	141	0.26	0.23 to 0.29	87						
	NO_3^-	0.17	-0.003 to 0.34	150	0.15	0.12 to 0.18	77						
	NH_4^++NO_3^-	0.15	-0.05 to 0.35	195				0.63	0.29 to 0.96	47	0.28	-0.06 to 0.62	45
N flux	Δ NH_4^+	-0.05	-0.31 to 0.20	50									
	Δ NO_3^-	0.45	0.21 to 0.69	79	0.42	0.28 to 0.57	27	0.49	-0.26 to 1.25	11			
	Δ NH_4^++NO_3^-	0.36	0.14 to 0.58	101	0.42	0.35 to 0.48	58	0.32	-0.12 to 0.8	25	0.74	0.22 to 1.26	23
Other	Soil N	0.53	0.31 to 0.75	187	0.18	0.15 to 0.20	88	0.57	0.28 to 0.72	103	0.07	-0.34 to 0.48	51
	Soil C:N	-0.05	-0.25 to 0.14	100				-0.01	-0.37 to 0.34	39			
	SOM	0.59	0.18 to 0.99	90				0.35	-0.12 to 0.83	26	0.4	-0.13 to 0.94	36

2.3.2 Invasive species traits

Of the four types of invasive species traits (leaf %N, leaf C:N, litter %N, and litter C:N) and nine soil N effect sizes, three trait-by-effect size correlations are significant (Table 3; Supplement: Figure 6). The mean trait values of invasive species are 2.3±0.1 leaf %N, 1.2±0.1 litter %N, 28.7±1.0 leaf C:N, and 54.2±4.7 litter C:N (Supplement: Figure 4). Invasive species with high leaf %N increase soil N in invaded areas to greater extents. Invasive species with high leaf %N and litter %N decrease soil C:N in invaded areas to greater extents. The quality of the CWM data included in each of the models with a significant fixed effect did not mediate the resulting invasion effect size and visual inspection of scatterplots did not reveal a quality bias (Supplement: Figure 6). Moreover, this was the case for all significant models that included CWM trait data (Supplement: Figure 7, Supplement: Figure 8).

2.3.3 Reference CWM traits

Of the four types of reference CWM traits and nine soil N effect sizes, two trait-by-effect size correlations are significant (Table 3; Supplement: Figure 7). The mean trait values of reference CWMs are 1.7±0.0 leaf %N, 1.0±0.1 litter %N, 31.6±1.2 leaf C:N, and 77.9±5.3 litter C:N (Supplement: Figure 4). Invasions that occur where the reference CWM leaf C:N values are high tend to increase mineralization and decrease soil C:N to greater extents.

2.3.4 Dissimilarity in CWM traits

Of the four types of CWM trait dissimilarities among invaded and reference area communities (Inv. – Ref.) and nine soil N effect sizes, eight trait-by-effect size correlations are significant (Table 3; Supplement: Figure 8). The mean differences in CWM trait values between invaded and reference communities are +0.38±0.04 and +0.19±0.07 for leaf and litter %N, and +0.84±1.15 and -17.47±4.13 for leaf and litter C:N, respectively (Supplement: Figure 4). Invasions in which the invaded community has much greater litter %N than the reference community increased ammonium and total inorganic N pools to greater extents. Invasions in which the invaded community has much lower litter C:N than the reference community increased ammonium, total inorganic N, and mineralization rates to greater extents. Lastly, invasions in which the invaded community has much lower leaf C:N than the reference community increased total inorganic N and mineralization rates and decreased soil C:N values to greater extents.

Table 3: Summary of mixed effects models used to explain invasion effect sizes (response variable) using reference community-weighted mean (CWM) trait values, invasive species' trait values, or dissimilarity between invaded and reference area CWM traits (Δ CWM) as fixed effects. Random effects account for variation within studies and among studies within the same article. For each model fit, the following data are reported: hypothesized relationship direction (Hyp.), fixed-effect coefficient (est), pseudo-R^2 (R_P^2), and number of studies (k). Models with fixed effect p-values less than 0.1 are highlighted in grey

Response variable		Trait type	Invasive species							Reference CWM							CWM Dissimilarity						
			Hyp.	Leaf			Litter			Hyp.	Leaf			Litter			Hyp.	Leaf			Litter		
				est	R_P^2	k	est	R_P^2	k		est	R_P^2	k	est	R_P^2	k		est	R_P^2	k	est	R_P^2	k
Inorganic N pool	NH_4^+	%N	+	0.01	0	122	0.56	0	19	-	-0.14	0	117	-0.11	0	26	+	0.19	0	115	0.61	0.45	26
	NO_3^-		+	0.07	0	128	-0.01	0	20	-	-0.04	0	124	0.01	0	30	+	0.11	0.02	122	0.18	0	30
	$NH_4^+ + NO_3^-$		+	0.09	0	173	0.51	0	21	-	-0.06	0	146	0.17	0	24	+	0.15	0.02	144	1.55	1	24
N flux	Δ NH_4^+		+	0.12	0	38	0.86	0.83	13	-	0.05	0	38	-0.53	0.54	15	+	-0.06	0	38	0.32	0.33	15
	Δ NO_3^-		+	0.14	0.06	67	0.81	0	16	-	0.13	0	65	-1.23	0	18	+	0.19	0.1	65	0.58	0	18
	Δ $NH_4^+ + NO_3^-$		+	0.01	0	84	0.04	0	15	-	-0.01	0	77	-0.04	0	18	+	0.21	0.05	77	-0.10	0	18
Other	Soil N		+	0.34	0.06	170	0.51	0.02	27	-	0.16	0.07	154	-0.04	0	28	+	-0.03	0	152	0.34	0	28
	Soil C:N		-	-0.20	0.14	89	-0.46	1	10	+	-0.05	0	81	0.27	0	12	-	0.01	0	81	-0.38	1	12
	SOM		+	0.27	0.05	88	0.11	0	19	-	0.15	0	81	-0.36	0	18	+	-0.03	0	81	0.51	0	18
Inorganic N pool	NH_4^+	C:N	-	-0.02	0.02	64	-0.02	0	11	+	0.01	0.05	48	0.01	0.01	17	-	-0.01	0.02	48	-0.02	0.15	17
	NO_3^-		-	0.00	0	75	-0.03	0	12	+	0.01	0	61	0.00	0	18	-	0.00	0	61	-0.02	0	18
	$NH_4^+ + NO_3^-$		-	-0.01	0	97	-0.01	0	14	+	0.01	0.02	70	0.01	0.04	23	-	-0.02	0.22	70	-0.02	0.59	23
N flux	Δ NH_4^+		-	0.01	1	19	-0.01	1	7	+	0.01	1	16	0.00	0	10	-	-0.01	0.68	16	0.00	0.59	10
	Δ NO_3^-		-	0.00	0	38	-0.01	0	14	+	0.00	0	28	0.01	0.11	18	-	0.00	0	28	-0.01	0.21	18
	Δ $NH_4^+ + NO_3^-$		-	0.00	0	59	-0.01	0	16	+	0.02	0	46	0.00	0.03	22	-	-0.03	0	46	-0.01	0.42	22
Other	Soil N		-	-0.01	0	101	-0.02	0	20	+	0.00	0	89	0.01	0.05	23	-	-0.01	0	89	-0.01	0.29	23
	Soil C:N		+	0.00	0	70	NA	NA	12	-	-0.02	0	66	0.00	0.37	13	+	0.03	0.04	66	0.01	0	13
	SOM		-	0.03	0.02	28	-0.03	0.27	13	+	0.02	0.14	22	0.00	0	13	-	-0.01	0	22	0.00	0	13

2.3.5 Presence of N-fixing species

Most observations do not include an N-fixing species in the reference plant community or as the focal invasive species (295 of 404 studies, Table 1). N-fixing species are present in reference area plant communities in 58 studies and of those studies, 20 studies include an invasive species that is also an N-fixing species (Table 1). A study's N-fixing status is a significant predictor for three soil nitrogen invasion effect sizes: total inorganic N, soil N, and soil C:N (Figure 1). For total inorganic N, studies with only resident N-fixers have significantly lower and negative effect sizes than studies without resident N-fixers. In fact, studies with N-fixers only in the reference plant community are the only studies to show a decrease in total inorganic N in invaded plots. For soil N, studies with only invasive N-fixers tend to have larger soil N effect sizes that other N-fixing status levels, but post-hoc tests reveal that the only levels that differ are studies with invasive species N-fixers only and studies without any N-fixers (Figure 1). For soil C:N, studies with only resident N-fixers have significantly higher soil C:N effect sizes than the other N-fixing status levels and higher soil C:N values in invaded than reference areas.

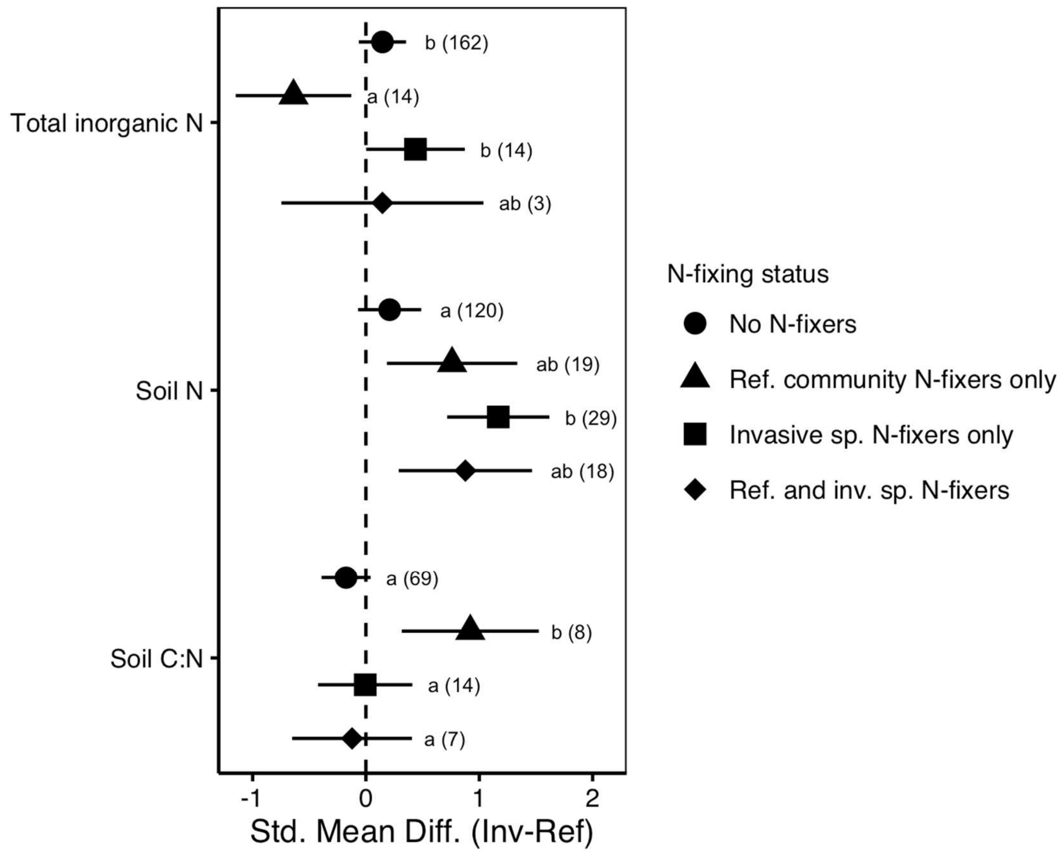

Figure 1: The presence of an N-fixing plant species modifies invasion impacts on total inorganic N, mineralization, soil N, and soil C:N effect sizes; mean +/- 95% confidence interval with the number of studies shown in parentheses. Different letters indicates that levels are significantly different according to post-hoc t-tests (alpha=.05). Effect sizes were calculated as the standard mean difference (Hedges' d) of invaded minus reference areas such that values >0 indicate greater values in invaded than reference areas

2.3.6 Study type

Observational field studies are by far the most frequent study type in this dataset (273 of 404 studies, Table 1). Study type is a significant predictor for nitrate and soil organic matter effect sizes (Figure 2). For nitrate effect sizes, all study designs except observational field studies exhibit neutral or slightly negative effect sizes, whereas observational field studies found significantly higher nitrate pools in invaded areas relative to reference areas (Figure 2). Soil organic matter values were marginally to significantly higher in invaded areas for studies that are experimental removals and greenhouse experiment study designs. Soil organic matter effect sizes in observational studies do not significantly differ based on invasion (Figure 2). Only one experimental addition study measured differences in soil organic matter in invaded and reference areas.

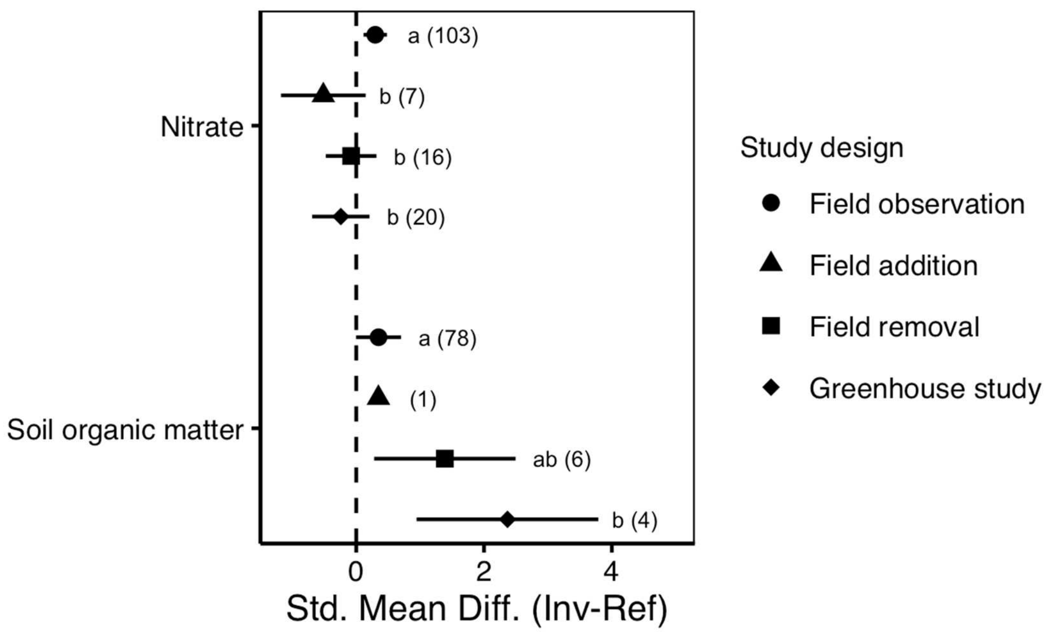

Figure 2: Study design mediates invasion effect sizes for nitrate and soil organic matter; mean +/- 95% confidence interval with the number of studies shown in parentheses. Different letters indicate significant differences among study designs according to post-hoc t-tests (alpha=.05). Effect sizes were calculated as the standard mean difference (Hedges' d) of invaded minus reference areas such that values >0 indicate greater values in invaded than reference areas

2.4 Discussion

Invasion impacts on soil N pools and fluxes are persistent and widespread across the current literature. While on average invasions increase soil N pools and fluxes, many invasion events have no impact or even reduce N cycling properties. For the first time in a global synthesis study, we show that plant traits that characterize plant species' nutrient uptake and release can, in part, explain why some invasive species cause large increases in soil N cycling and why some invaded communities are more likely to experience these changes. Invasive species with higher leaf and litter %N increase soil N and decrease soil C:N in invaded areas to greater extents. Moreover, we found that dissimilarity in CWM traits among invaded and reference plant communities performed better than raw CWM trait values at explaining variability in soil N cycling. For example, invasions that fostered the greatest increases in ammonium, total inorganic N, and mineralization rates were invasions in which the invaded community has much greater litter %N and lower litter C:N than the reference community. We advocate for using plant traits and metrics that most closely reflect the mechanisms with which species alter soil N cycling to address the challenge of predicting the ecosystem consequences of invasions

Invasions are associated with increases in inorganic N pools, nitrification and mineralization rates, and total soil N on average. These results fit with previous meta-analyses conducted by Liao *et al.* (2008), Vilà *et al.* (2011), and Castro-Díez *et al.* (2014), and confirm that plant invasions are correlated with a syndrome of nitrogen cycling changes characterized by larger inorganic N pools and faster N transformation rates. Our synthesis added 107 new experimental studies with 33 studies that fit into a new category of study design: experimental addition of invasive species. These studies support the finding that invasions are associated with altered N cycling, which alleviates some concerns that the "invasion impact" signal is a cause, rather than a consequence, of invasion. In addition, our synthesis added 189 new studies of non-N-fixing invasive species and, unlike previous syntheses, our dataset includes studies (studies (k) =16 studies) where the invasive species is a non-N-fixing species and the reference community contains an N-fixing species. The fact that adding studies with non-N-fixing invasive species and studies with N-fixing species present in the reference communities are *still* associated with altered N cycling, bolsters evidence that invasion causes shifts in N cycling outside of the limited context of N-fixing invasive species that enter communities without an N-fixing species.

When apparent, associations between invader traits and impacts fit our predictions. Greater invasion impacts were observed when the invasive species had N-rich trait values (e.g. high %N and low C:N), whereas more modest shifts in N cycling occurred where the reference community had N-rich CWM trait values. Only two invasive species' plant traits (leaf and litter %N) explained variation in the magnitude and direction of invasion impacts on soil N and soil C:N. In these cases where invader traits could explain invasion effect sizes on soil properties, our hypotheses regarding the direction of the relationship were supported. The varying explanatory power of different traits for different effect sizes is not surprising given the variation in data coverage among trait types. The trait that was most widely reported in the literature, invasive species' leaf %N, explained variation in bulk soil properties like soil N and soil C:N. At this point, we cannot distinguish whether our inability to detect the effects of other plant traits on N cycling is a result of the small sample size or an actual lack of effects. Particular traits also may be more or less integral to the ecosystem function of interest (Díaz *et al.* 2004), and the particular traits that are integral for a species in a given environment may not be the same traits that are integral in the context of a different invasion. For example, *Bromus tectorum*'s leaf %N may be a more important driver of its impact on soil inorganic N than that of *Morella faya*, since *M. faya*'s status as an N-fixing species is more closely associated with its soil N impacts. In addition, some of the ways in which invasive plant species alter soil N cycling may not have been well characterized by the traits included in this study. Belowground plant traits may have more explanatory power for some invasive species and contexts, but are far more rarely measured or reported (Bardgett, Mommer & de Vries 2014). And finally, another reason that some traits and effects sizes may not be strongly correlated is because of the role of abiotic factors that mediate soil N processes and thus may have obscured the expected relationship between plant nutrient-use traits and soil N values (Castro-Díez *et al.* 2014; Hobbie 2015).

There is limited evidence that reference community characteristics besides N-fixing status strongly shape invasion impacts on N cycling. As previous studies have found, invasion impacts on N cycling are diminished where N-fixing species are already present (Vilà *et al.* 2011; Castro-Díez *et al.* 2014). However, we did not find that reference CWM trait values alone were very strong predictors of invader effects on N cycling. With the exception of reference CWM leaf C:N, most reference species traits did not have sufficient information to explain variation in invasion effects on soil N. In line with our hypotheses and general findings regarding the influence of N-fixing context, invasion was associated with larger impacts on mineralization and smaller declines in soil C:N in cases where reference plant communities had high CWM leaf C:N. Whereas leaf and litter %N were the informative invasive species' traits, the ratio of C:N better explains the role of reference community plant traits in mediating plant invasion effect magnitude and direction. This finding could suggest that plant tissue stoichiometry is a more important indicator of ecosystem sensitivity to soil N cycling perturbations than plant tissue N concentrations alone. Overall, reference community plant tissue traits do not perform well at explaining variability in invasion impacts, and this might be because reference plant communities can shape N cycling through many other pathways, including by altering abiotic conditions that mediate N transformations.

Dissimilarity in leaf and litter traits among invaded and reference communities explained the magnitude and direction of the most types of invasion impacts on soil N (i.e. pools, fluxes, and bulk soil properties). Trait dissimilarities were able to capture most of the trends detected by invasive species' traits and reference community traits, while in addition, explaining variation in inorganic N flux effect sizes. This result supports the idea, identified by previous reviewers (Ehrenfeld 2003; Castro-Díez *et al.* 2014), that invasive species can have both direct and indirect impacts on N cycling by having dissimilar characteristics and potentially by displacing native plant species that are likewise important in shaping N pools and fluxes. To calculate the changes in plant trait values that result from invasion, we used an approach that closely reflects the way that invasion impacts on ecosystem processes are calculated. Some field studies evaluate leaf and litter plant CWM traits in invaded and reference areas to match soil N measurements and analyses, but until now, there has never been a global-scale synthesis to evaluate the explanatory power of this information. Our findings suggest that dissimilarity between how invaded and reference plant communities take up and release nutrients, approximated by leaf and litter traits, is critical to understanding variability in invasion impacts on N cycling.

On the whole, the influence of study design on the magnitude and direction of invasion effect sizes was limited with only two of nine effect sizes mediated by study design. This fits with the findings of Castro-Díez *et al.* (2014) in that study design does not appear to strongly mediate detection of invasion impacts on soil N cycling. But it is important to note that both Castro-Díez *et al.* (2014) and this study detected a few ways that soil N cycling measures were sensitive to study design. We found that invasions are associated with larger soil nitrate pools in observational studies but not experimental studies, whereas Castro-Díez *et al.* (2014) found no difference in invasion effect sizes on soil N pools and fluxes based on whether the study was observational, experimental, or a combination. Our finding might suggest that the impact of invasion on nitrate pools develops only after a long lag period since observational studies are more likely to capture an older invasion than manipulative experiments. Another possibility, not mutually exclusive from the previous one, is that high nitrate concentrations promote invasion and are not a consequence of invasion.

In contrast to Castro-Díez's finding that removal studies have smaller N pool and flux effect sizes than studies with a non-invaded reference (Castro-Díez *et al.* 2014), we found that soil organic matter effect size was the only impact type that was sensitive to experimental removal studies. We found that invaded areas are associated with more soil organic matter in experimental removal and greenhouse studies than observational studies. This finding is supported by only a few studies, but may suggest that experimental removal disrupts soil organic matter whereas greenhouse studies involve extensive root proliferation in invaded pots that is difficult to separate from the soil matrix. Alternatively, we may only be able to detect the impact of invasion on soil organic matter in highly controlled studies such as field and greenhouse experiments. More work is needed to understand why invasion appears to increase soil organic matter in only these study design types. A variety of study designs are needed to understand how invasions influence and are influenced by nitrate and soil organic matter, but on the whole, researchers should be pleasantly surprised to know that observational studies do provide similar insights to invasion impacts as experimental studies.

Further research is needed to address the particular mechanisms that lead to invasion effects on soil N and how these mechanisms may differ among invasive species and among invaded plant communities. Our findings regarding the relationship (or lack thereof) between plant traits and effect size magnitude and direction points to the need to better understand why some plant characteristics are better than others at explaining changes in target ecosystem properties. Last, we recommend considering a measure of functional trait dissimilarity to determine the magnitude and direction that an ecosystem process with shift in response to a change in community composition. Furthering this research will contribute to our ability to predict and plan for the impact of plant invasions on nutrient cycling in many contexts.

2.5 Supplemental material

2.5.1 Equations

Equation 1: Hedges' d

Hedge's d for a given study was calculated as

$$d = \frac{(\bar{X}_{inv} - \bar{X}_{ref})}{S} J$$

where $\bar{X}_{inv}$ and $\bar{X}_{ref}$ are soil measurement means from invaded and reference areas, respectively, for each study. S is the pooled standard deviation and J is a weighting factor based on the number of replicates such that

$$J = 1 - \frac{3}{4(N_{inv} + N_{ref} - 2) - 1}.$$

The variance for Hedges' d was calculated as

$$var(d) = \frac{N_{inv} + N_{ref}}{N_{inv} N_{ref}} + \frac{d^2}{2(N_{inv} + N_{ref})}.$$

Equation 2: Pseudo-R^2

A pseudo-R^2 was calculated as the proportional reduction in the total variance,

$$\frac{(\sigma_{red.,article}{}^2 + (\sigma_{red.,study}{}^2) + (\sigma_{full,article}{}^2 + (\sigma_{full,study}{}^2)}{(\sigma_{red.,article}{}^2 + (\sigma_{red.,study}{}^2)}$$

where $\sigma_{red.,article}{}^2$ and $\sigma_{red.,study}{}^2$ are the variances associated with the nested random effects, article and study, from a reduced model that does include fixed effects. The terms $\sigma_{full,article}{}^2$ and $\sigma_{full,study}{}^2$ are the variances associated with the nested random effects from the full model.

2.5.2 Tables

Table 4: List of the 143 articles used in our meta-analysis on the impacts of invasion on soil N cycling. If the article appears in a previous meta-analysis on this topic, there is a 'Yes' in one of the three previous meta-analysis columns: Liao 2008, Vilà 2011, and Castro-Díez 2014. For inclusion in this meta-analysis, at least one study within each article needed to have provided data for one of the nine soil N measurement types listed. The number of studies per soil N measurement type that were extracted from each article is shown below

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	Number of studies (k) per soil N measurement type								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
Vitousek	1989	Yes			3	3	0	0	3	3	0	0	0
Witkowski	1991	Yes	Yes	Yes	2	2	2	0	0	0	2	0	2
Musil	1993	Yes	Yes	Yes	0	0	0	0	0	0	1	0	0
Wedin	1993	Yes	Yes		0	0	0	0	0	6	6	6	0
McIntosh	1995	Yes			0	0	0	0	0	0	3	3	3
Stock	1995	Yes	Yes		2	2	2	0	0	2	2	2	2
Vinton	1995				0	0	0	0	0	6	6	6	0
Asner	1996	Yes	Yes		0	0	2	2	2	0	2	0	0
Schlesinger	1996				0	0	1	0	0	0	0	0	0
Mitchell	1997	Yes		Yes	8	8	8	0	0	0	0	0	8
Boswell	1998	Yes			0	0	0	0	0	0	1	1	1
D'Antonio	1998		Yes	Yes	1	1	0	0	0	0	0	0	0
Templer	1998				2	0	0	0	0	0	0	0	2
Christian	1999	Yes	Yes	Yes	0	0	2	0	0	0	2	2	0
Kourtev	1999				5	5	0	5	5	5	5	0	0
Maron	1999			Yes	2	2	0	0	0	2	2	2	2
Meyerson	1999	Yes	Yes		4	0	0	0	0	0	2	0	0
Otto	1999	Yes	Yes		2	0	0	0	0	2	2	0	0
Saggar	1999	Yes	Yes		0	0	1	0	0	0	1	1	0
Belnap	2001	Yes	Yes	Yes	2	2	0	0	0	0	2	0	0

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	Number of studies (k) per soil N measurement type								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
Ehrenfeld	2001	Yes	Yes	Yes	7	7	7	7	7	0	0	0	2
Evans	2001	Yes	Yes	Yes	0	0	2	0	0	2	0	0	0
Mack	2001			Yes	0	0	0	0	0	2	0	0	0
Scott	2001	Yes	Yes	Yes	0	0	3	0	0	0	3	3	0
Svejcar	2001	Yes	Yes		3	3	3	3	3	3	3	0	0
Blank	2002	Yes	Yes		0	0	0	0	0	0	1	1	0
Hoopes	2002	Yes	Yes		2	2	2	0	0	0	0	0	2
Booth	2003	Yes	Yes	Yes	1	1	2	1	2	1	2	0	0
Kourtev	2003	Yes		Yes	2	2	0	2	2	2	0	0	2
Mack	2003	Yes		Yes	0	0	0	0	4	4	4	4	0
McCarron	2003	Yes			1	1	1	0	0	1	0	0	0
Monaco	2003				0	10	0	0	0	0	0	0	0
Windham	2003	Yes	Yes		3	3	0	3	3	3	3	0	3
Haubensak	2004		Yes	Yes	0	0	0	0	1	1	1	1	0
Haubensak	2004		Yes	Yes	0	0	0	0	0	0	1	0	0
Hook	2004	Yes	Yes		0	0	3	0	0	9	9	9	0
Lett	2004	Yes			0	0	0	0	0	0	2	2	0
McCulley	2004	Yes			3	3	3	0	3	3	3	3	0
Rice	2004		Yes	Yes	0	1	0	0	1	1	1	0	1
Standish	2004	Yes	Yes	Yes	1	1	0	0	0	0	0	0	0
Suding	2004		Yes		0	0	1	0	0	1	0	0	0
Symstad	2004				0	0	0	0	0	0	2	0	0
Wolf	2004		Yes		1	1	0	1	1	0	0	1	0
Yelenik	2004			Yes	0	0	0	1	1	1	1	0	1
Ashton	2005	Yes		Yes	1	1	1	0	0	0	0	0	1
Belnap	2005	Yes	Yes	Yes	2	2	2	2	0	0	2	0	2
Funk	2005	Yes	Yes	Yes	0	0	1	0	1	1	0	0	0
Hagos	2005				0	0	0	0	0	0	1	0	1
Hawkes	2005	Yes		Yes	2	2	2	2	2	0	0	0	0

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	**Number of studies (k) per soil N measurement type**								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
Hughes	2005	Yes	Yes		0	0	3	0	0	0	0	0	0
Lindsay	2005	Yes	Yes	Yes	1	1	1	0	0	0	1	0	0
Reed	2005	Yes	Yes	Yes	0	0	0	0	2	2	2	2	0
Vanderhoeven	2005	Yes	Yes	Yes	0	0	0	0	0	0	8	8	8
Yu	2005				1	1	0	0	0	0	1	0	0
Allison	2006	Yes	Yes		0	0	0	0	0	0	3	0	0
Baer	2006	Yes	Yes		1	1	1	1	1	1	1	1	0
Caldwell	2006	Yes	Yes	Yes	0	0	0	0	0	0	1	1	1
Chapuis-Lardy	2006	Yes	Yes	Yes	0	0	0	0	0	0	3	3	0
Cheng	2006				0	0	0	0	0	0	1	1	1
Domenech	2006		Yes		0	0	0	0	0	0	1	1	0
Fickbohm	2006	Yes	Yes		2	2	2	0	2	2	0	0	2
Fisher	2006		Yes		0	0	0	0	0	0	2	0	0
Heneghan	2006	Yes	Yes	Yes	0	0	3	2	2	2	3	3	0
Leary	2006				0	0	0	0	0	0	2	0	0
LeJeune	2006				1	1	0	0	1	1	1	1	0
Li	2006	Yes			3	3	3	0	0	0	3	0	3
Mahaney	2006	Yes			3	3	3	0	0	0	0	0	3
Prevosto	2006				3	3	0	0	0	0	3	3	0
Rimer	2006	Yes			0	0	1	0	0	1	0	0	0
Sperry	2006	Yes	Yes		4	4	4	0	0	0	4	0	0
Liao	2007				0	0	0	0	0	0	2	2	0
Nosshi	2007			Yes	0	0	6	0	0	6	6	6	0
Taniguchi	2007				0	0	0	0	0	0	6	6	0
Yelenik	2007		Yes		2	2	0	0	0	0	2	0	2
Blank	2008		Yes		0	0	2	0	0	2	2	2	0
DeCant	2008			Yes	0	0	1	0	0	0	1	0	1
Hooker	2008				2	2	0	0	0	0	2	2	0
Kurten	2008		Yes		1	0	0	0	0	1	1	1	0

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	Number of studies (k) per soil N measurement type								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
Malcolm	2008				0	0	0	0	4	4	4	0	4
Marchante	2008		Yes	Yes	1	1	1	0	1	0	1	1	0
Rodgers	2008		Yes	Yes	0	0	5	0	0	0	0	0	0
Adams	2009				1	1	1	0	0	0	0	0	0
Castro-Díez	2009		Yes	Yes	2	2	2	2	2	2	0	0	2
Chen	2009		Yes		1	1	0	0	1	1	1	1	0
Martin	2009		Yes	Yes	0	0	0	0	0	1	1	1	1
McGrath	2009				0	4	0	0	0	0	0	0	0
Peltzer	2009			Yes	4	4	0	0	0	0	4	0	0
Rossiter-Rachor	2009			Yes	1	1	0	1	1	0	1	0	0
Rout	2009				0	2	0	0	0	0	0	0	0
Sanon	2009				0	0	0	0	0	0	1	0	0
Scharfy	2009			Yes	1	1	1	1	1	1	1	1	0
Sharma	2009			Yes	0	0	1	1	1	1	1	1	0
Zhang	2009			Yes	1	1	0	0	0	0	1	0	0
Adair	2010				1	1	1	0	0	0	0	0	0
Aguilera	2010				0	0	0	1	1	1	1	1	0
Blank	2010				17	17	17	0	0	17	0	0	0
DeMeester	2010				1	1	1	1	1	1	0	0	0
Fan	2010				0	0	1	0	0	0	1	0	1
HilleRisLambers	2010				0	0	30	0	0	0	0	0	0
Shaben	2010			Yes	1	1	1	0	0	0	2	2	0
Yelenik	2010				0	0	4	2	2	2	0	0	2
Zhang	2010				0	0	0	0	0	0	3	3	0
Archibald	2011				1	1	0	0	0	0	1	0	0
Corbin	2011			Yes	4	4	4	0	4	4	0	0	0
Elgersma	2011				0	0	0	2	2	0	0	0	0
Gaertner	2011				0	0	3	0	0	0	3	0	0
Hellmann	2011				1	1	1	0	0	0	0	0	1

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	Number of studies (k) per soil N measurement type								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
McGlone	2011				1	1	0	0	0	0	1	0	0
Mitchell	2011				0	0	0	0	0	2	0	0	0
Peng	2011				1	1	1	1	1	0	0	0	0
Perkins	2011				0	0	24	0	0	0	0	0	0
Thorpe	2011				3	3	0	0	1	0	0	0	0
Timsina	2011				0	0	0	0	0	0	2	0	2
Turner	2011				4	4	4	0	0	0	0	0	4
Abella	2012				1	1	1	0	0	0	1	0	1
Bottollier-Curtet	2012				1	1	1	0	0	0	0	0	1
Lee	2012				4	4	4	4	4	4	0	0	1
McEwan	2012				3	3	3	0	0	0	3	3	0
Morris	2012				0	0	0	0	0	1	1	1	1
Ruffner	2012				0	0	2	0	0	2	2	2	0
Sanon	2012				0	0	0	0	0	0	1	0	1
Schaeffer	2012				0	0	2	2	2	2	2	2	0
Boudiaf	2013				0	0	0	0	0	0	2	2	2
DeMarco	2013				0	0	0	0	0	0	2	2	0
Dickens	2013				3	3	0	0	0	0	3	3	0
Gasch	2013				1	1	0	0	0	0	1	1	0
Hagan	2013				4	4	4	0	0	0	4	0	4
Hamman	2013				0	0	4	0	0	0	0	0	0
Hickman	2013			Yes	1	1	0	0	1	1	0	0	0
Irl	2013				0	0	0	0	0	0	0	1	0
Jaeger	2013				1	1	0	0	0	0	1	1	0
Lyons	2013				0	0	0	2	2	0	0	0	0
Rout	2013				0	2	0	0	0	0	0	0	0
Tharayil	2013				1	1	0	0	0	1	0	0	0
Von Holle	2013				2	2	0	0	0	0	0	0	0
Yelenik	2013				0	0	0	0	0	1	0	0	0

Author	Year	Liao 2008	Vilà 2011	Castro-Díez 2014	Number of studies (k) per soil N measurement type								
					NH_4^+	NO_3^-	NH_4^++ NO_3^-	Δ NH_4^+	Δ NO_3^-	Δ NH_4^++ NO_3^-	Soil N	Soil C:N	SOM
Bansal	2014				0	2	2	0	0	0	0	0	0
Cusack	2014				1	1	1	0	0	0	1	1	0
Dickens	2014				2	2	2	2	2	0	1	1	0
Dickens	2014				3	3	3	0	2	0	2	2	1
Price	2014				1	1	0	0	0	0	0	0	0
Staska	2014				0	3	0	0	0	0	3	3	0
Yu	2014				0	0	12	0	0	0	12	0	12

Table 5: Quality of CWM trait data is summarized below by community type and trait type. CWM values that were reported within the study have the highest quality with a quality rank of four. CWM value estimates that were calculated with more than 25% of the species in the community having the following attributes are considered higher in quality: a) measured species' cover data (as opposed to cover estimate based on author's description), b) species-specific cover data (as opposed to assuming species are present in equal abundance, c) species-specific trait values that are either reported within the original study or extracted from TRY

Comm. type	CWM calc'd	More than 25% of species in the community have...				Qual. rank	Number of CWM values				CWM values (%)			
		Measured cover	Sp.-specific cover	Reported traits	Sp.-specific TRY traits		Leaf %N	Litter %N	Leaf C:N	Litter C:N	Leaf %N	Litter %N	Leaf C:N	Litter C:N
Invasive sp.	No	NA	NA	NA	NA	4	48	8	10	7	13.2	16.0	4.8	14.9
	Yes	y	y	y	NA	3	14	6	11	6	3.8	12.0	5.3	12.8
		y	y		y	3	26	0	1	2	7.1	0.0	0.5	4.3
		y	y			2	12	0	6	0	3.3	0.0	2.9	0.0
		y			y	2	6	0	0	0	1.6	0.0	0.0	0.0
			y	y	NA	2	45	19	39	14	12.4	38.0	18.8	29.8
			y		y	2	144	0	60	13	39.6	0.0	28.8	27.7
		y				1	0	2	4	0	0.0	4.0	1.9	0.0
			y			1	48	10	58	4	13.2	20.0	27.9	8.5
				y	NA	1	2	3	0	1	0.5	6.0	0.0	2.1
					y	1	19	0	15	0	5.2	0.0	7.2	0.0
						0	0	2	4	0	0.0	4.0	1.9	0.0
Invaded area	No	NA	NA	NA	NA	4	26	24	14	20	7.6	36.4	6.6	33.3
	Yes	y	y	y	NA	3	16	6	12	7	4.7	9.1	5.7	11.7
		y	y		y	3	26	0	1	0	7.6	0.0	0.5	0.0
		y	y			2	7	0	6	0	2.0	0.0	2.8	0.0
		y		y	NA	2	4	0	1	0	1.2	0.0	0.5	0.0
		y			y	2	9	0	0	1	2.6	0.0	0.0	1.7
			y	y	NA	2	37	15	34	11	10.8	22.7	16.0	18.3
			y		y	2	133	0	55	13	38.9	0.0	25.9	21.7

Comm. type	CWM calc'd	More than 25% of species in the community have...				Qual. rank	Number of CWM values				CWM values (%)			
		Measured cover	Sp.-specific cover	Reported traits	Sp.-specific TRY traits		Leaf %N	Litter %N	Leaf C:N	Litter C:N	Leaf %N	Litter %N	Leaf C:N	Litter C:N
		y				1	2	2	4	1	0.6	3.0	1.9	1.7
			y			1	30	10	55	4	8.8	15.2	25.9	6.7
				y	NA	1	6	7	3	2	1.8	10.6	1.4	3.3
					y	1	46	0	23	1	13.5	0.0	10.8	1.7
						0	0	2	4	0	0.0	3.0	1.9	0.0
Reference area	No	NA	NA	NA	NA	4	25	24	14	20	7.8	40.7	9.0	37.0
	Yes	y	y	y	NA	3	16	5	12	6	5.0	8.5	7.7	11.1
		y	y		y	3	23	0	0	0	7.2	0.0	0.0	0.0
		y	y			2	5	0	5	0	1.6	0.0	3.2	0.0
		y		y	NA	2	4	1	0	1	1.3	1.7	0.0	1.9
		y			y	2	9	0	1	1	2.8	0.0	0.6	1.9
			y	y	NA	2	32	12	23	8	10.0	20.3	14.8	14.8
			y		y	2	94	0	9	0	29.4	0.0	5.8	0.0
		y				1	4	0	4	1	1.3	0.0	2.6	1.9
			y			1	25	5	47	6	7.8	8.5	30.3	11.1
				y	NA	1	15	8	14	5	4.7	13.6	9.0	9.3
					y	1	59	1	21	2	18.4	1.7	13.5	3.7
						0	9	3	5	4	2.8	5.1	3.2	7.4

Table 6: Overall impact of invasion on soil N cycling based on this dataset is summarized below with associated publication bias checks and Q statistics. See Methods for a description of the fail-safe number, correlation among effect and sample size, and Q statistics

Soil measurement		Grand effect size			Fail-safe number		Correlation: Effect and sample size			Q statistics			
		Effect size (d^+)	95% CI	k	num.	p-val	r	slope coef.	p-val	Q_E	Q_E p-val	Q_M	Q_M p-val
Inorganic N pool	NH_4^+	0.26	0.12 to 0.40	141	1827	<.001	0.01	-0.01	ns	307.9	<.001	13.4	<.001
	NO_3^-	0.17	-0.003 to 0.34	150	314	<.01	-0.17	-0.01	ns	405.2	<.001	3.7	0.054
	NH_4^++NO_3^-	0.15	-0.05 to 0.35	195	404	<.01	-0.06	-0.01	ns	492.2	<.001	2.0	0.153
N flux	Δ NH_4^+	-0.05	-0.31 to 0.20	50	0	ns	0.07	0.01	ns	74.5	0.011	0.2	0.699
	Δ NO_3^-	0.45	0.21 to 0.69	79	1321	<.001	-0.33	-0.03	ns	167.0	<.001	13.4	<.001
	Δ NH_4^++NO_3^-	0.36	0.14 to 0.58	101	1143	<.001	-0.19	-0.07	0.046	207.9	<.001	10.0	0.002
Other	Soil N	0.53	0.31 to 0.75	187	8416	<.001	-0.01	-0.01	ns	608.3	<.001	21.7	<.001
	Soil C:N	-0.05	-0.25 to 0.14	100	0	ns	0.06	0.01	ns	190.7	<.001	0.3	0.586
	SOM	0.59	0.18 to 0.99	90	1508	<.001	0.05	-0.02	ns	368.5	<.001	8.0	0.005

2.5.3 Figures

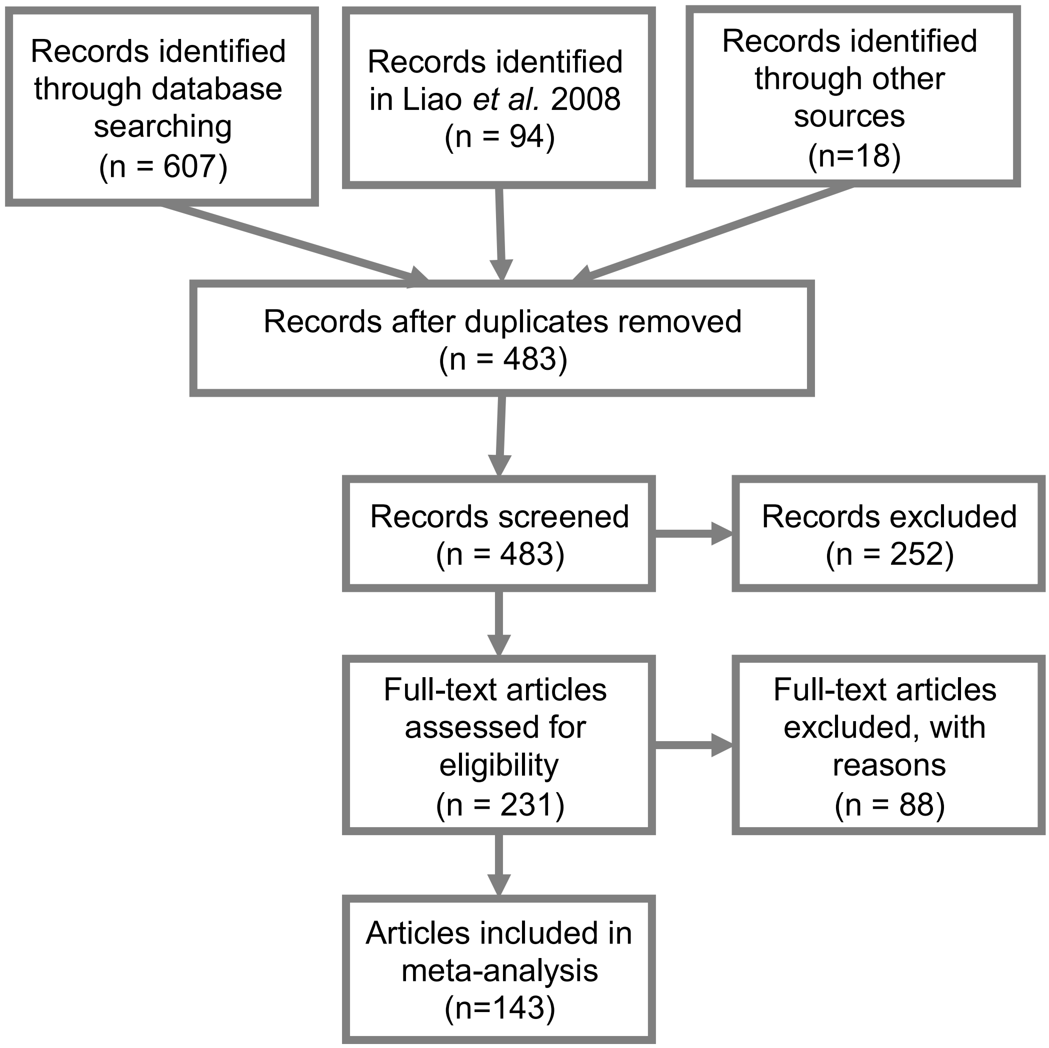

Figure 3: Flow chart of the article identification and selection process based on the PRISMA 2009 flow diagram based on Moher *et al.* 2009

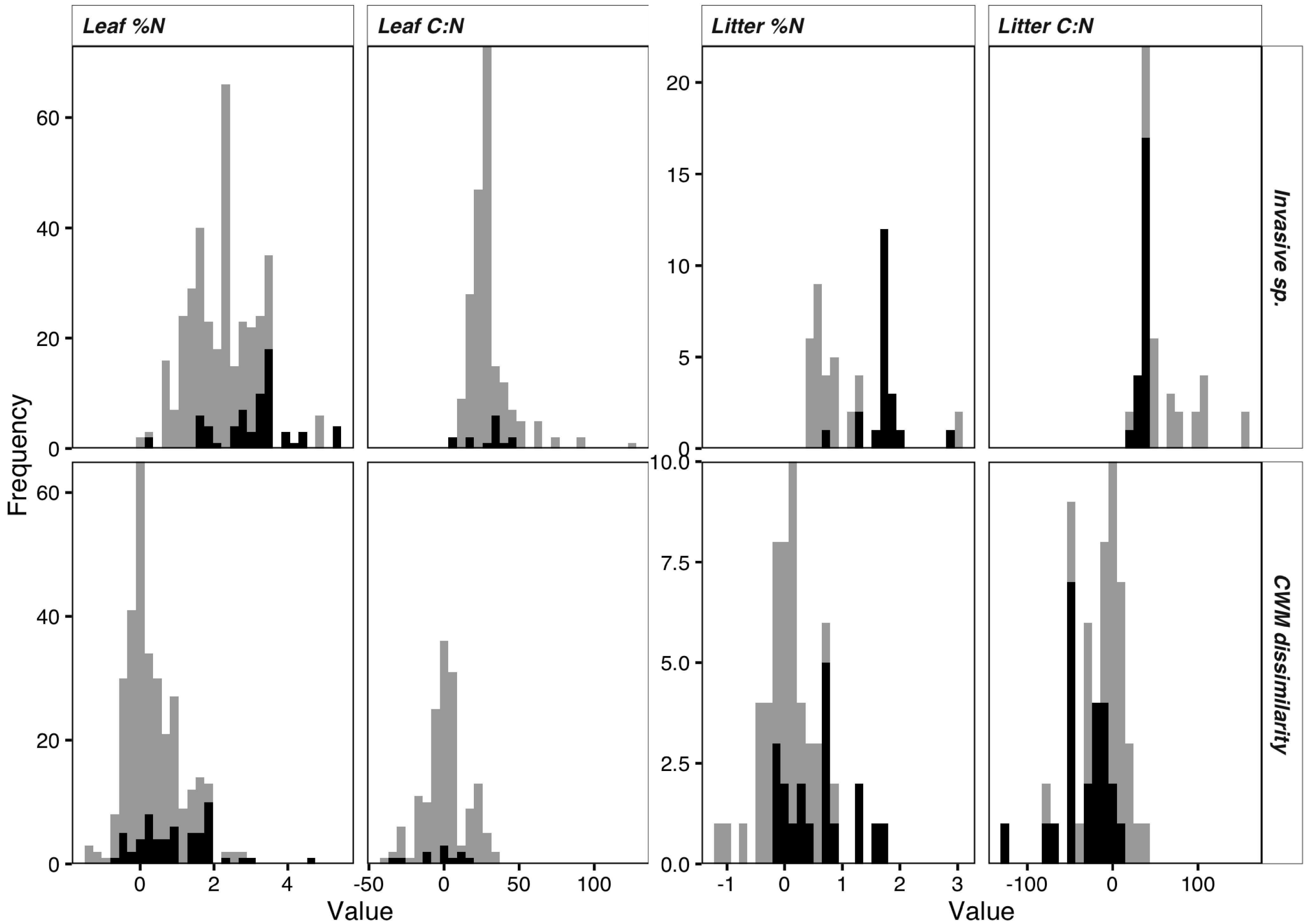

Figure 4: Frequency distribution of invasive species' trait values and CWM trait dissimilarities (Inv.-Ref.). Studies with an N-fixing invasive sp. are shown in black fill bars, whereas gray fill bars represent studies with non-fixing invasive species.

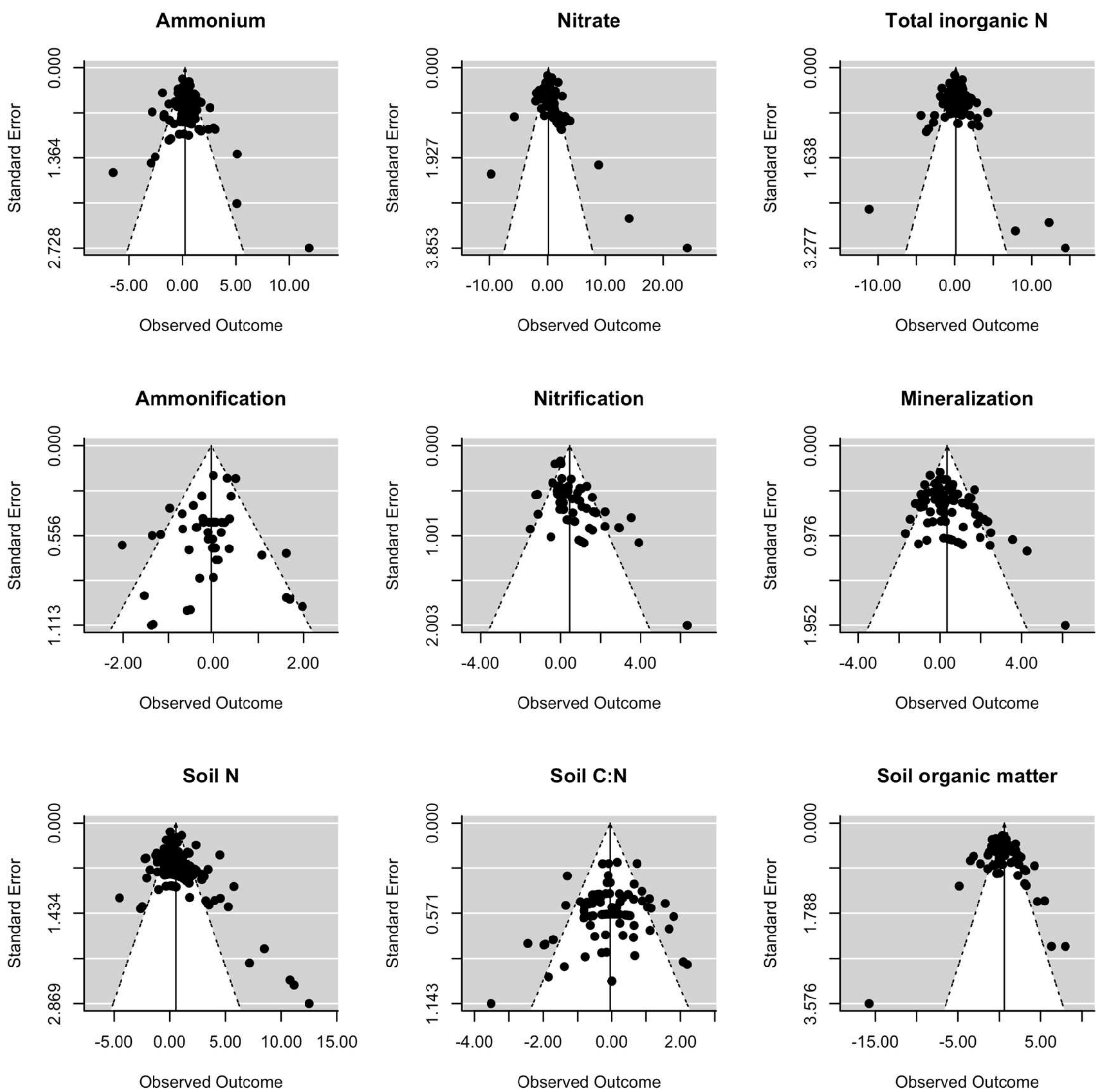

Figure 5: Funnel plots for all effect size measures. A symmetrical funnel shape relationship between study effect sizes and standard errors suggests that there is no publication bias toward either large positive or negative effect sizes

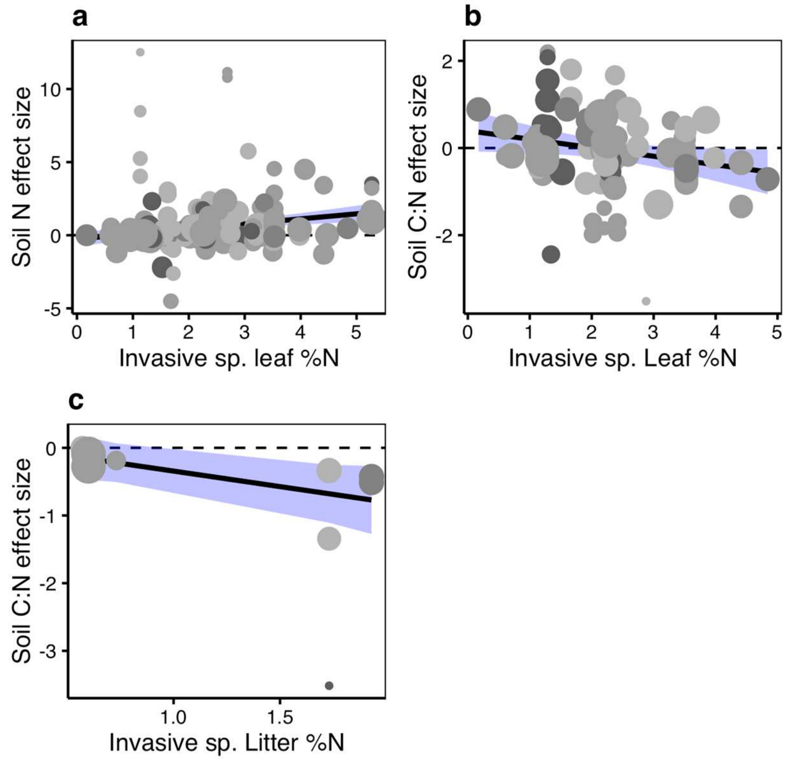

Figure 6: Invasive species' trait values correlate with study effect sizes for a subset of trait types and effect size measures. Leaf %N correlates with a) soil N, b) soil C:N, and c) Litter %N correlates with soil C:N effect sizes. Point size is scaled by variance in the study's effect size such that larger points have smaller variance. Point shade represents CWM quality rank such that darker points are higher in quality. Solid line and blue shaded region show the meta-regression model fit and the 95% confidence interval. Dashed y=0 line aids in distinguishing between studies with positive (higher values in invaded area) and negative (lower values in invaded area) effect sizes

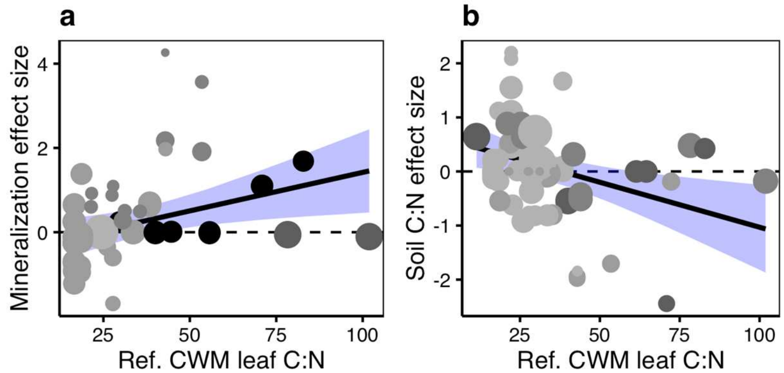

Figure 7: Reference CWM trait values correlate with study effect sizes for a subset of trait types and effect size measures. Leaf C:N correlates with a) mineralization and b) soil C:N effect sizes. Refer to Figure 6 caption for a full description of plot attributes

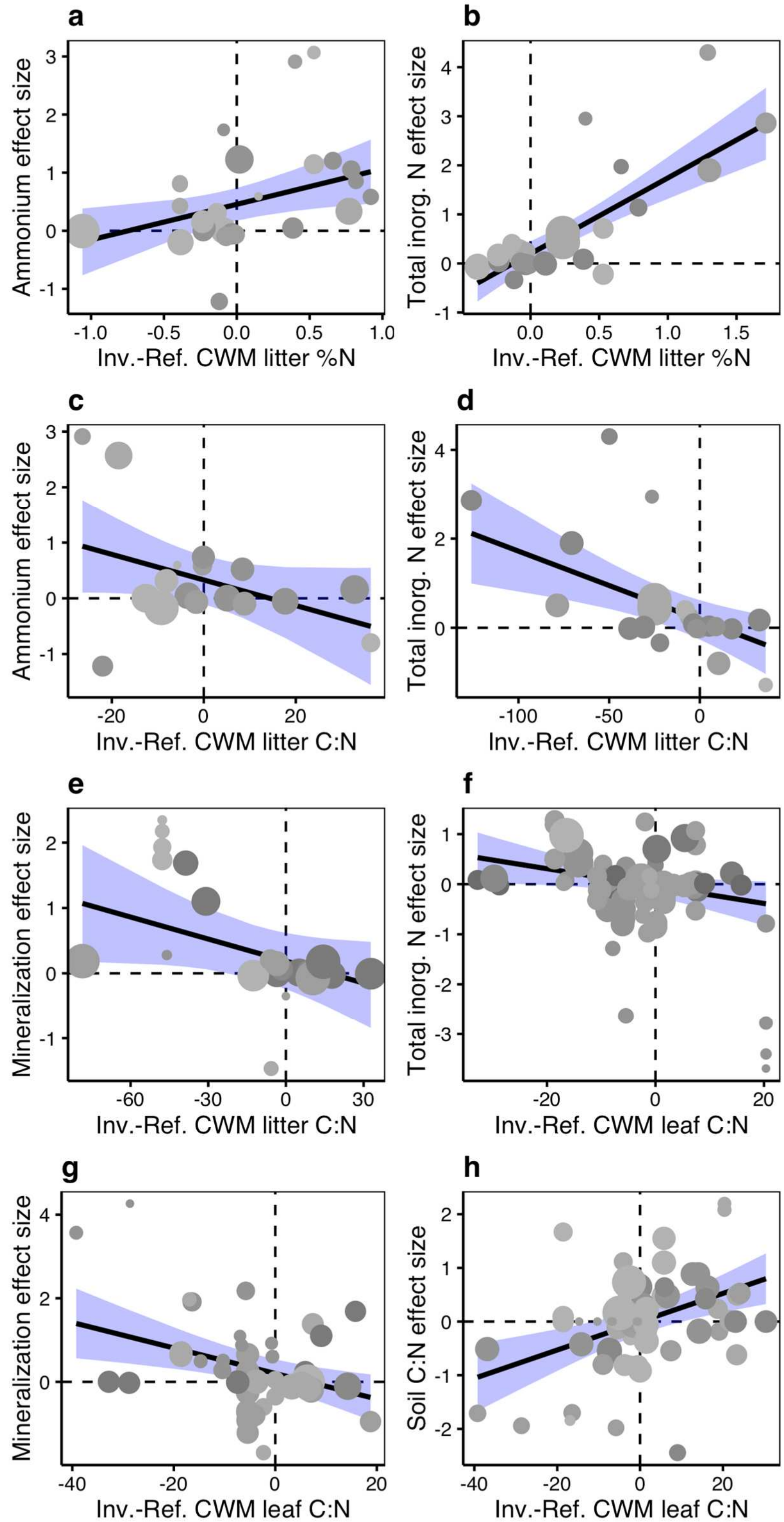
a
Ammonium effect size
Inv.-Ref. CWM litter %N
b
Total inorg. N effect size
Inv.-Ref. CWM litter %N
c
Ammonium effect size
Inv.-Ref. CWM litter C:N
d
Total inorg. N effect size
Inv.-Ref. CWM litter C:N
e
Mineralization effect size
Inv.-Ref. CWM litter C:N
f
Total inorg. N effect size
Inv.-Ref. CWM leaf C:N
g
Mineralization effect size
Inv.-Ref. CWM leaf C:N
h
Soil C:N effect size
Inv.-Ref. CWM leaf C:N

Figure 8: CWM trait dissimilarities correlate with study effect sizes for a subset of trait types and effect size measures. Dissimilarity in litter %N correlates with a) ammonium and b) total inorganic N effect sizes. Dissimilarity in litter C:N correlates with c) ammonium, d) total inorganic N, and e) mineralization effect sizes. Dissimilarity in leaf C:N correlates with f) total inorganic N, g) mineralization, and h) soil C:N effect sizes. Refer to Figure 6 caption for a full description of plot attributes

3. Neighbor identity can be a strong determinant of an invader's impact on soil moisture and nitrogen availability

3.1 Introduction

Plant species invasions can dramatically alter ecosystem processes. However, our ability to predict the direction and magnitude that invasions alter ecosystem processes is lacking. One reason for this knowledge gap is the lack of data on the relationship been the invader's density and its impact. Another reason is that sampling designs rarely consider the importance of co-occurring species that may differentially mediate an exotic species' impact on an ecosystem process. In this study, we measured the impact of varying densities of an invasive grass on soil nitrogen (N) pools and fluxes and evaluated how the following co-occurring conditions, neighboring plant presence and identity, mediate that impact.

Regulation of soil nitrogen (N) pools and transformations is an important ecosystem function and there is growing evidence that plant invasions can significantly alter soil N pools and fluxes (Liao *et al.* 2008). Many of these studies evaluated invader impacts in paired plots where the introduced species is observationally or experimentally present and absent (Parker *et al.* 1999; Ehrenfeld 2003). However, it is important to consider invader density and co-occurring conditions to enhance our understanding of the processes by which these plants alter N cycling.

Paired plot studies do little to inform our understanding of the shape of the relationship between invader abundance and impact. While this relationship is often assumed to be linear for the purpose of management (Parker *et al.* 1999; Ricciardi 2003), there are real constraints on ecosystem processes that are important to consider for the purpose of evaluating and predicting invasion impacts. Net N mineralization, for example, is a microbial-mediated process that is limited by microbial nutrient demands and physical soil conditions such as pH and moisture. In addition, maximum soil inorganic N concentrations are limited by the soil's texture and cation-exchange capacity.

Recent studies within invasion biology have also highlighted the importance of understanding how species interactions contribute to invader impacts on ecosystem processes (Ricciardi *et al.* 2013; Hickman *et al.* 2013; Kuebbing & Nuñez 2015). Invasive species can have indirect impacts on N cycling by displacing native plant species that are important in shaping N pools and fluxes. It is also well-known that co-occurring species can act synergistically to impact ecosystem processes such as soil N pools and fluxes (Finzi & Canham 1998; Hooper *et al.* 2005).

To explore the influence of co-occurring species on the impact an invasive plant species has on soil N, we evaluated the role of invader density and neighbor identity on soil N pools and transformations using the invasive grass, *Microstegium vimineum*. Like many invasive plant species (Liao *et al.* 2008), *Microstegium* tends to increase soil N pools and the rate of soil N transformations (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Kourtev, Ehrenfeld & Haggblom 2003; Adams & Engelhardt 2009; DeMeester & deB Richter 2010; Lee *et al*. 2012). More specifically, in both observational and experimental studies *Microstegium* has been associated with elevated soil moisture (Adams & Engelhardt 2009; Lee *et al.* 2012), nitrate concentrations (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Adams & Engelhardt 2009; DeMeester & deB Richter 2010; Lee *et al.* 2012), and net potential nitrification rates (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Kourtev *et al.* 2003; Lee *et al*. 2012). Although the exact mechanisms by which *Microstegium* alters soil N remains unknown, it is generally established that novel plant species can impact soil nitrogen pools and transformations by altering the quality or quantity of plant N uptake and release, and the activity or composition of microbial N-transformers (Knops *et al.* 2002; Hawkes *et al.* 2005; Chapman *et al.* 2006).

Many invasive plants can enter a wide-range of habitat types, varying in species identity and abundance, making the role of these factors on invader impacts important to investigate. The invasive species, *Microstegium*, readily invades both species-rich fields and depauperate forest understories (Cole & Weltzin 2004; Warren, Wright & Bradford 2010). There is evidence that *Microstegium* increases inorganic N pools and fluxes in both habitat types (Ehrenfeld *et al.* 2001; DeMeester & deB Richter 2010), but it is unclear whether one should expect the effects of *Microstegium* on N cycling to vary depending on the identity of co-occurring species. This problem is complicated by the fact that changes in neighborhood identity are concurrent with changes in key environmental factors controlling N cycling, such as soil moisture, texture, and resource availability. In this study, we use a highly simplified system to address the role of the neighboring plants as mediators of *Microstegium*'s impact on soil N in a controlled greenhouse environment.

The objective of this study was to evaluate (1) how *Microstegium* abundance affects soil N pools and fluxes and soil moisture and (2) how neighbor plant species with differing functional effects on nitrogen transformations might mediate these relationships. To determine the role of invader density and neighbor context on *Microstegium*'s soil N impact, we manipulated the density of *Microstegium* in the absence and presence of either *Panicum virgatum*, which naturally co-occurs with *Microstegium* in its invasive range, or *Sorghum bicolor*, which produces nitrification-inhibiting compounds (Zakir *et al.* 2008). We expected that soil nitrate and nitrification would increase with higher absolute and relative abundance of *Microstegium* and anticipated that these relationships may be non-linear. Moreover, we expected that *Microstegium* would increase nitrification to a greater extent when grown with a nitrification-inhibiting species than with a neighbor with no known effect on nitrification. These results would suggest that both the abundance of the invader and the presence and identity of co-occurring species are important in this simple system for predicting the magnitude and direction that soil measures will change with increasing invader abundance.

3.2 Methods

3.2.1 Experimental Design

To experimentally determine the impact of *Microstegium* density, neighbor presence, and neighbor identity, on soil conditions, a varying number of *Microstegium* individuals were grown in 3.79-liter greenhouse pots (16 cm in diameter) in the absence or presence of *Panicum* or *Sorghum* (see experimental design, Figure 9). Five levels of *Microstegium* density treatment (0, 1, 2, 4, 5 *Microstegium* individuals) were crossed factorially with 3 neighbor treatment levels (No neighbor, *Panicum*, or *Sorghum*). Together with an end-member *Microstegium* density treatment of 6 *Microstegium* individuals, this created 16 treatments that were replicated 10 times and organized on a greenhouse bench in blocks.

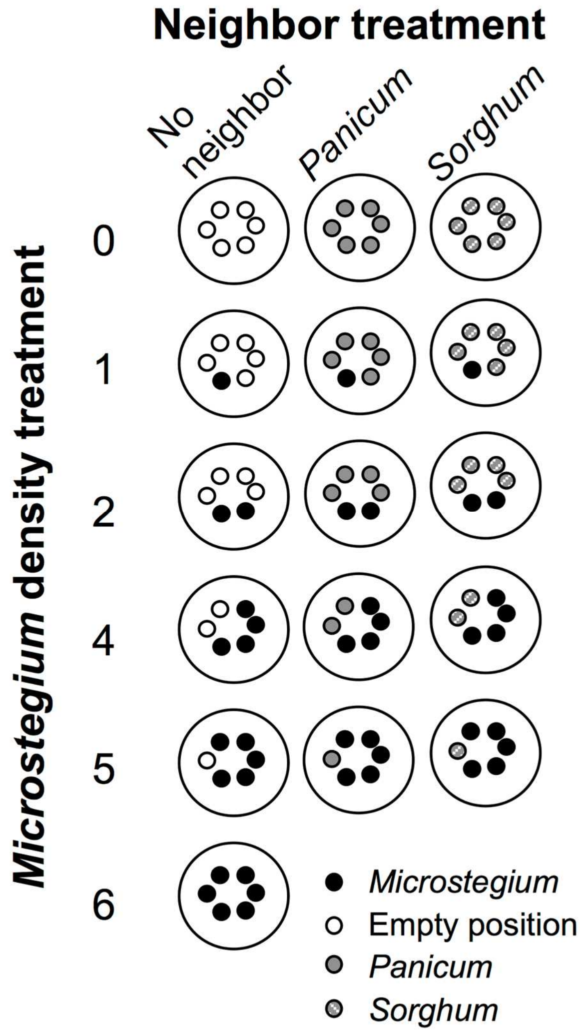

Figure 9: Experimental design consisting of 16 treatments: 5 *Microstegium* density levels (0, 1, 2, 4, 5, 6 individuals) and 3 neighbor treatment levels (No neighbor, *Panicum*, *Sorghum*). Each pot (large circles) contained 6 positions for a *Microstegium* individual (black circle), empty position (white circle), *Panicum* (gray circle) or *Sorghum* individual (gray circle with diagonal pattern). Treatments were replicated 10 times and organized in blocks

Microstegium individuals were transplanted as seedlings with 1 true leaf from a natural field invasion in Duke Forest, Durham, NC. *Panicum* and *Sorghum* were grown from seed (*Panicum* from Prairie Moon Nursery, Winona, MN, and *Sorghum* from

Granite Seed Co., Lehi, UT) and then transplanted into experimental pots when approximately 10-15 cm tall. Individual seedlings were added to experimental pots in June 2011 with their position fully randomized and mapped for identification at harvest time. Each of the 6 positions within a pot (Figure 9) had a 5 g plug of field-collected soil sieved through 4 mm mesh to introduce a soil community representative of soils that *Microstegium* typically invades. Field soil was collected from an upland hardwood forest in May 2011 (Duke Forest, Durham, NC) where *Microstegium* is locally absent but present within 100 m. The overall soil matrix consisted of Fafard 3B soilless potting media (2:1:1 v/v peat moss, pine bark, perlite/vermiculite; Sun Gro Horticulture, Agawam, MA). Experimental pots were located inside a fenced enclosure adjacent to the Duke University Greenhouse. Pots received full sun and experienced approximately 14 hr. days, 74% relative humidity, and high and low temperatures of 33°C and 21°C, respectively. Pots were watered as needed by Duke University Greenhouse staff. Plant and soil material were collected with permission from the Duke Forest Teaching and Research Laboratory at Duke University, Durham, NC.

3.2.2 Laboratory analyses

Ten weeks after the experiment was established, all aboveground plant material was harvested, sorted by species, dried to a constant mass, and weighed. Soil was sieved through 2-mm mesh. Soil moisture was measured gravimetrically using standard methods. Inorganic N (NH_4^+ and NO_3^-) was extracted immediately from soil samples with 2 M KCl (10:1) and measured with an auto-analyzer (Lachat; Hach, Loveland, CO). Net N mineralization rates (ammonification, nitrification, mineralization) were estimated by extracting soil inorganic N from samples after incubating soils (2.5 g fresh weight) aerobically in the laboratory at 22 °C for 12 days. Net N mineralization rates are presented on a per day basis. Soil moisture was not adjusted prior to the incubations so that the influence of differences in soil moisture across samples could be captured. Incubating soils were covered in punctured Parafilm in order to minimize soil moisture loss while allowing for aeration.

3.2.3 Statistical analyses

To evaluate the role of density manipulations on species biomass and total pot biomass across neighbor treatments, variation in dry aboveground plant biomass was analyzed with mixed linear effects models that had *Microstegium* density treatment as the fixed effect and block as the random effect.

Differences in total dry aboveground plant biomass and soil measures among unplanted pots and species monocultures were analyzed with mixed linear effects models that had block as a random effect.

To evaluate the role of *Microstegium* biomass on soil measures in the absence of neighbors, variation in each soil measure from *Microstegium*-only pots was analyzed with mixed linear effects models that had *Microstegium* biomass as a fixed effect and block as a random effect. To explore the shape of these relationships, *Microstegium* biomass was also included as a quadratic term.

To evaluate the role of *Microstegium* biomass, neighbor presence and identity, and their interaction on soil measures, variation in each soil measure from all pots was analyzed with mixed linear effects models that had *Microstegium* biomass, neighbor treatment, and their interaction as fixed effects and block as a random effect. To explore the shape of these relationships, *Microstegium* biomass was also included as a quadratic term. To explore the role of total plant biomass as a driver of variation in soil measures, a second model also included linear and quadratic total dry aboveground biomass terms.

Soil measures included total dry aboveground plant biomass per pot, soil N pools (ammonium, nitrate, total inorganic N), soil N fluxes (ammonification, nitrification, mineralization), and soil moisture. All statistical analyses were conducted using R Statistical Software (R Core Team 2014). The package *lme4* was used to construct and analyze the linear mixed effects models (Bates *et al.* 2015). The significance of fixed effects was determined using the Satterthwaite approximation to estimate parameter degrees of freedom as implemented by the package *lmerTest* (Kuznetsova, Brockhoff & Christensen 2014).

3.3 Results

3.3.1 Plant biomass

Microstegium biomass increased with greater *Microstegium* density treatments and was suppressed in the presence of neighboring species (Figure 10, Supplement: Table 7). The presence of *Sorghum* suppressed *Microstegium* biomass to a greater extent than *Panicum* (Figure 10). Total aboveground plant biomass per pot was highest in pots where *Sorghum* was present since Sorghum has a much greater *per capita* biomass than the other plant species (Figure 10, Supplement: Table 7). As such, there was a negative relationship between *Microstegium* density and total plant biomass when *Sorghum* was assigned as the neighbor treatment; whereas when *Panicum* was assigned as the neighbor treatment, total biomass did not change across *Microstegium* density treatments (Figure 10, Supplement: Table 7).

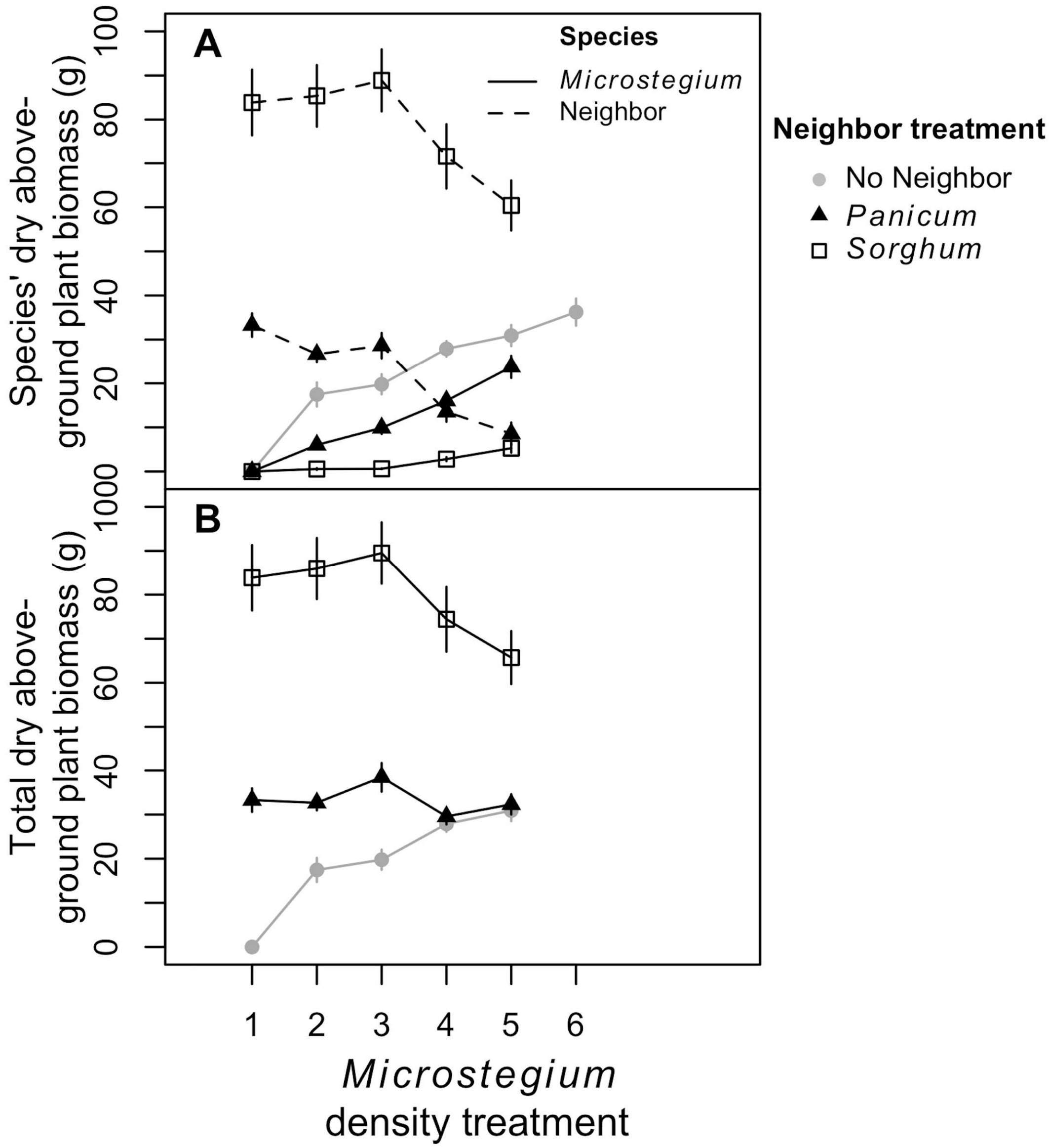

Figure 10: Effect of density and neighbor treatments on plant biomass. Variation in a) species' dry aboveground biomass (g), and b) total dry aboveground biomass (g) are shown. All biomass responses varied by density and neighbor treatment and their interaction (p<.01). Bars are (mean±SE) (n=10)

3.3.2 Inorganic N pools

In the absence of plant inorganic N uptake, unplanted pots had the highest soil inorganic N concentrations (Figure 11, Supplement: Table 8). Plant species did not differ in how they mediated soil ammonium concentrations; however, concentrations under *Sorghum* tended to be the lowest (Figure 11). Relative to ammonium, nitrate constituted the majority of the total inorganic N pool across treatments (Figure 11, Figure 12). Nitrate and total inorganic N concentrations tend to be highest in *Microstegium*, intermediate in Panicum, and lowest in *Sorghum* monocultures (Figure 11).

In the absence of neighbors, increases in *Microstegium* biomass resulted in no change in ammonium concentrations (Figure 12, Supplement: Table 9). Soil nitrate and total inorganic N concentrations were suppressed by more *Microstegium* biomass at low biomass levels, e.g. less than 30 grams per pot; however, soil nitrate and total inorganic N concentrations were maintained or even increased with even greater increases in *Microstegium* biomass (Figure 12, Supplement: Table 9). The quadratic relationships between *Microstegium* biomass and nitrate concentrations held even when data in which *Microstegium* biomass equaled zero were excluded (linear term -2.00 ± 0.84, $p = 0.02$, quadratic term 0.04 ± 0.02, $p = 0.01$); the same was true for total inorganic N concentrations (linear term -1.92 ± 0.91, $p = 0.04$, quadratic term 0.04 ± 0.02, $p = 0.03$).

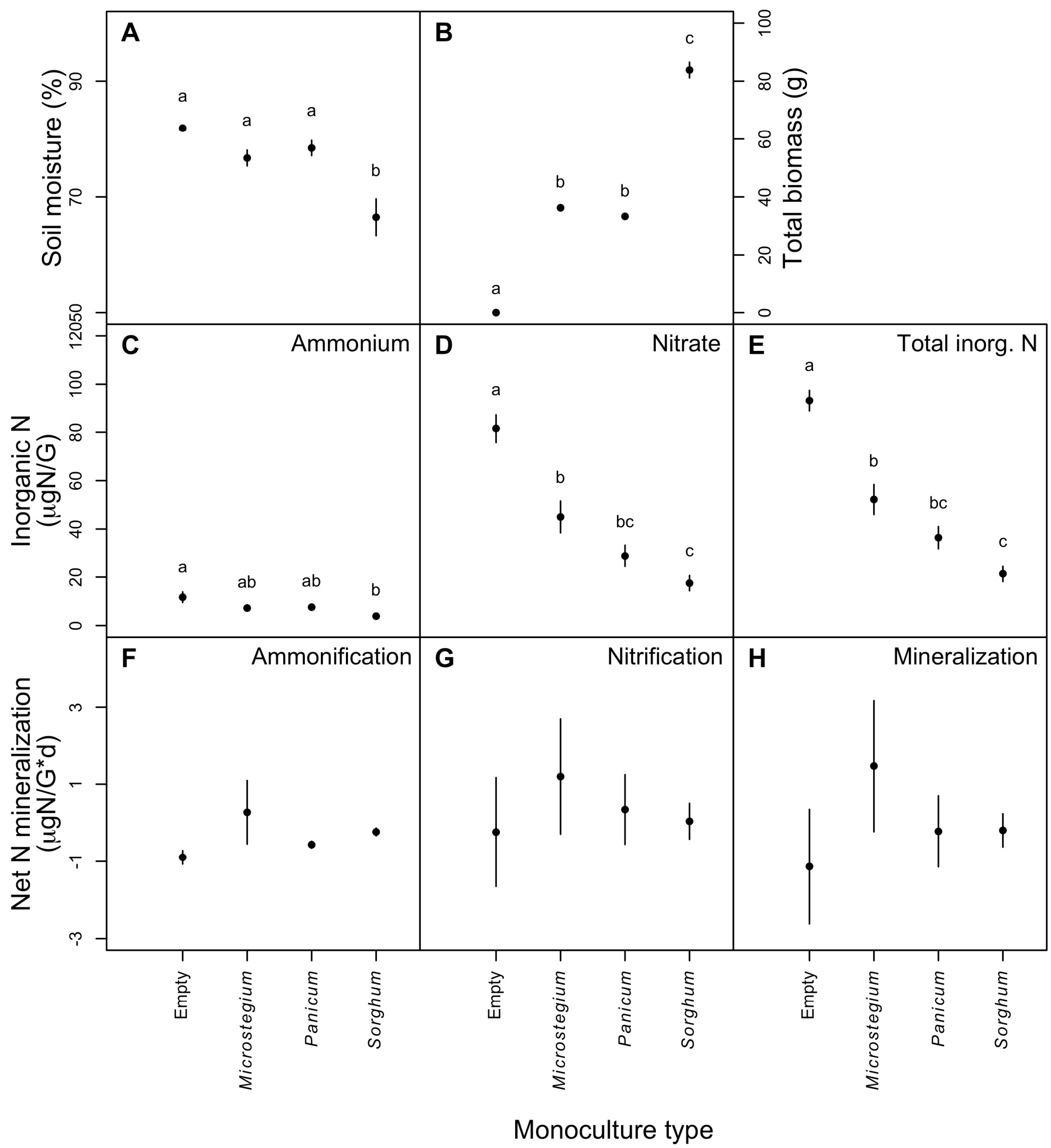

Figure 11: Effects of species monocultures and unplanted pots on soil measures and total dry aboveground plant biomass. Soil measures include a) ammonium, b) nitrate, and c) total inorganic N pools (µgN/G), d) ammonification, e) nitrification, and f) mineralization rates (µgN/G×d), and g) soil moisture (%). Total dry above ground plant biomass (g) is shown in panel h. All responses expect for N fluxes varied by plant type (p<.01). Bars are (mean±SE) (n=10) and different letters show significant differences according to post-hoc Tukey tests

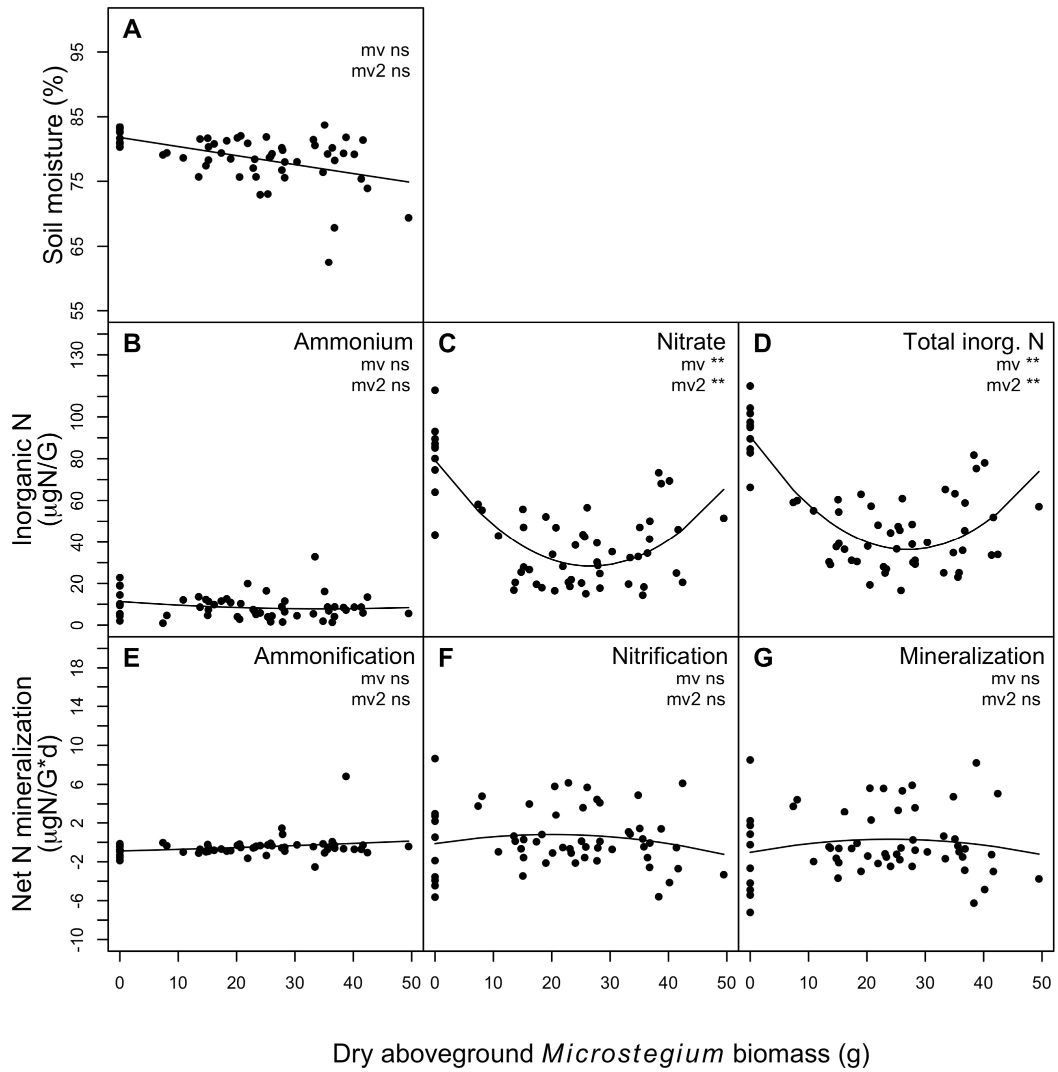

Figure 12: Effect of *Microstegium* biomass (g) on soil measures in the absence of neighbors. Soil measures are the same as in Figure 11. Text in the upper right corner of each panel indicates the significance of fixed effects where 'mv' and 'mv2' are linear and quadratic *Microstegium* biomass terms, respectively (ns = not significant, * = p<.05, ** = p<.01). Trendlines of fitted mixed effect model regressions are shown

Across *Microstegium* density treatments, all inorganic N concentrations (ammonium, nitrate, and total inorganic N) were lower in the presence of a neighbor and especially in the presence of *Sorghum* (Figure 13). Increasing *Microstegium* biomass and decreasing *Sorghum* density resulted in increases in nitrate and total inorganic N concentrations. Inorganic N concentrations, however, were not sensitive to changes in *Microstegium* biomass for pots that either did not contain a neighboring species or contained *Panicum* individuals (Figure 13). Soil nitrate and total inorganic N concentrations in pots with *Panicum*, in the same fashion as pots without neighboring species, were suppressed by more *Microstegium* biomass at low biomass levels, e.g. less than 30 grams per pot; however, these soil N concentrations were maintained or even increased with even greater increases in *Microstegium* biomass (Figure 13, Supplement: Table 10).

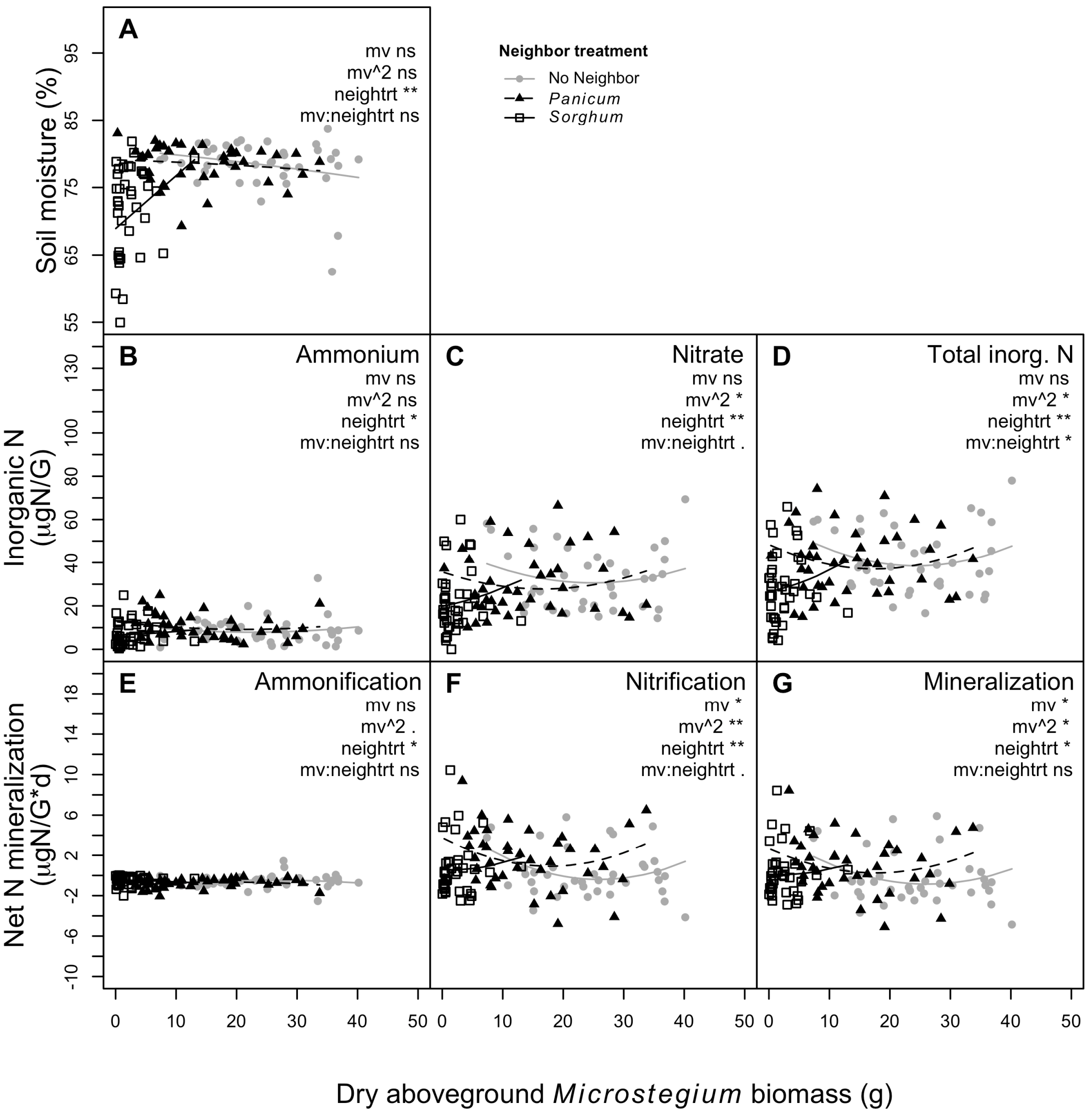

Figure 13: Effects of *Microstegium* biomass (g) on soil measures. Soil measures are the same as in Figure 11. Dataset only includes treatments in which *Microstegium* is growing with neighbors. Text in the upper right corner of each panel indicates the significance of fixed effects where 'mv' and 'mv2' are linear and quadratic *Microstegium* biomass terms, 'neigtrt' is neighbor treatment, and 'int' is the mv × neigtrt interaction (ns = not significant, * = p<.05, ** = p<.01). Data point symbols and colors differ by neighbor treatment. Trendlines of fitted mixed effect model regressions are shown

Nitrate and total inorganic N concentrations tended to decrease with increasing plant biomass, regardless of the species, but this did not hold true for patterns of ammonium concentrations (Figure 14, Supplement: Table 11).

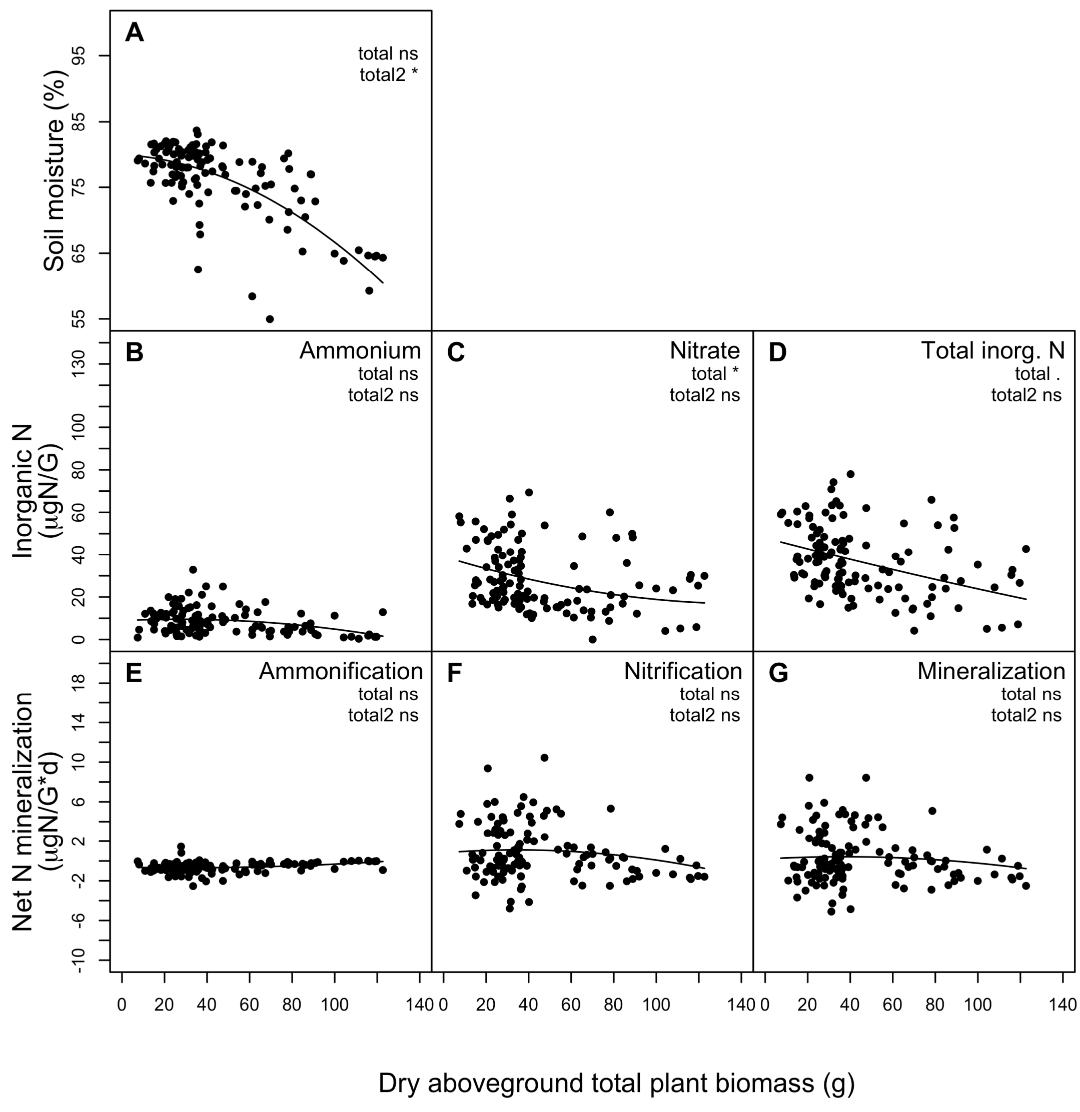

Figure 14: Effects of total dry aboveground plant biomass on soil measures. The data shown includes only treatments in which *Microstegium* is growing with neighbors. Text in the upper right corner of each panel indicates the significance of fixed effects where 'total' is total plant biomass (ns = not significant, * = p<.05, ** =

p<.01). Data point symbols and colors differ by neighbor treatment. Trendlines of fitted mixed effect model regressions are shown

3.3.3 N mineralization rates

None of the measured net N fluxes (ammonification, nitrification, mineralization) differed among species monocultures or unplanted pots (Figure 11, Supplement: Table 8) and none of the net N fluxes varied with *Microstegium* biomass in the absence of neighboring species (Figure 12, Supplement: Table 9). However, net N fluxes were sensitive to presence and identity of neighboring species (Figure 13, Supplement: Table 10). Net ammonification rates were slightly more negative, suggesting net ammonium immobilization, in pots with *Panicum* than when *Microstegium* was grown alone or with *Sorghum* (Figure 13). In addition, net nitrification and mineralization rates were more positive, suggesting net nitrate mobilization, in pots with *Panicum* than when *Microstegium* was grown alone or with *Sorghum* (Figure 13). A curvilinear response to *Microstegium* biomass, especially in the presence of *Panicum,* was also detected where net nitrification and mineralization rates tended to plateau or even slightly increase again under high levels of *Microstegium* biomass (Figure 13),

No relationship was detected between total plant biomass and the measured net N fluxes (Figure 14, Supplement: Table 11).

3.3.4 Soil moisture

Soil moisture did not differ among unplanted pots, and *Microstegium* and *Panicum* monocultures; however, it was significantly lower in *Sorghum* monocultures

(Figure 11, Supplement: Table 8). No relationship was detected between *Microstegium* biomass and soil moisture (Figure 12, Supplement: Table 9). Soil moisture was suppressed with increasing total plant biomass (Figure 14, Supplement: Table 11), such as in pots neighboring species, and particularly in the presence of *Sorghum* (Figure 13). After a threshold of plant biomass (approximately 50g) is met (Figure 14, Supplement: Table 11), soil moisture decreases rapidly.

3.4 Discussion

Contrary to previous studies, we did not find strong evidence that *Microstegium* biomass increases nitrate, net nitrification, or other net N mineralization rates when grown alone or with neighbors. More plant biomass, regardless of its species identity, drove decreases in soil moisture and inorganic N concentrations among our experimental manipulations of invader biomass, neighbor presence and identity. However, neighbor identity mediated net N mineralization rates in ways that could not be described by total plant biomass alone. As such, the results of our study serve as a cautionary tale against using solely a linear factor of invader density to estimate its impact on soil properties, particularly across areas where neighbor identity varies.

Microstegium has been associated with elevated nitrate concentrations, and net nitrification rates, and soil moisture (Ehrenfeld *et al.* 2001; Lee *et al.* 2012), but these patterns received only mixed support in this study based on monoculture comparisons and manipulation of *Microstegium* biomass in the absence of neighbors. Variation among monoculture and unplanted pots supported the pattern of higher nitrate concentrations under *Microstegium* but did not support findings for nitrification nor soil moisture. Net nitrification rates under *Microstegium* tended to be greater than under the other species' monocultures and in unplanted pots, but this difference was not significant. Soil organic matter can be an important mediator of soil N pools and fluxes by controlling substrate availability and microbial activity (Schimel & Bennett 2004; Laungani & Knops 2012). The soil substrate that was used in this study was high in soil organic matter (~65%), which likely acted to buffer changes in N pools and fluxes that we could have expected from experimental variation in plant rhizosphere extent and kind. Although *Microstegium* has been found to promote nitrification (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Kourtev *et al.* 2003; Lee *et al.* 2012) and *Sorghum* is known to suppress nitrification (Zakir *et al.* 2008), these patterns were not borne out in this study in the most extreme case of monoculture comparisons. It follows then that net nitrification rates did not vary with experimental changes in *Microstegium* density. The other result that ran contrary to our expectations was that soil moisture under *Microstegium* was not greater than the ecologically relevant plant species, *Panicum*. Given that paired plot observational studies have found higher soil moisture in areas invaded by *Microstegium* (Adams & Engelhardt 2009; Lee *et al.* 2012), our finding points to the idea that *Microstegium* may preferentially colonize microsites with high soil moisture.

In the context of neighbors, we did not find strong evidence in support of previously recorded *Microstegium* impacts on soil properties either. Increases in *Microstegium* in the presence of neighbor treatments did not correspond to linear increases in nitrate or significant decreases in soil moisture. In fact, increases in *Microstegium* biomass corresponded to net decreases in net nitrification and mineralization.

3.4.1 Neighbor presence, identity, and total plant biomass

Neighbor presence and identity, was an important predictor of all soil measures: soil inorganic N pools, N mineralization fluxes, and soil moisture. However, the role of neighbor identity cannot be fully disentangled from that of total aboveground biomass since the *per capita* biomass of *Sorghum* was much greater than *Panicum* and, by definition, pots that received the no neighbor treatment had the least total aboveground biomass, especially at low *Microstegium* densities. This is especially important to consider when interpreting the role of *Microstegium* biomass and neighbor treatments on soil measurements such as nitrate and total inorganic N concentrations and soil moisture, because total plant biomass was able to explain a significant portion of variation in these soil measures. For example, all inorganic N pool concentrations and soil moisture tended to be lower in the presence of a neighbor, especially in the presence of *Sorghum*. Moreover, the relationship between *Microstegium* biomass and these soil measures differed by neighbor treatment. For nitrate and total inorganic N pools, pots with *Sorghum* experienced increases in nitrate and total inorganic N concentrations with changes in *Microstegium* biomass, whereas pots without a neighbor and with *Panicum* did not differ much in inorganic N concentrations with changing *Microstegium* biomass. The role of total plant biomass, however, cannot be ruled out as an important underlying driver of the observed patterns.

Unlike inorganic N pools, N mineralization fluxes were not significantly correlated with total plant biomass. Thus, the findings that nitrification and mineralization rates tended to be higher in pots where *Microstegium* was grown with *Panicum* than *Sorghum* may be more indicative of species differences than differences in total plant biomass. No interactive effects, however, were detected *Microstegium* biomass and neighbor treatment on N mineralization rates.

3.4.2 Species interactions

One piece of evidence based on net nitrification measures suggests that neighboring species were more than simply the strong actors in this study. An intriguing synergism was observed between *Microstegium* and *Panicum* – faster nitrification rates were observed under *Microstegium* and *Panicum* mixtures than under *Microstegium* or *Panicum* alone. While the underlying mechanism remains unknown, this result points to the importance of considering neighbor identity when evaluating an invader’s impact.

3.4.3 Summary

In conclusion, invader density mediated nitrate concentrations in a curvilinear fashion and did not affect net nitrification rates or soil moisture as anticipated. Overall, the strong actors appeared to be the neighboring species instead of the invasive species, *Microstegium*. The experimental displacement of neighboring individuals with high per capita production and resource-use with *Microstegium* individuals, likely produced the observed phenomenon that soil moisture and nitrate concentrations increased with increasing invader density. In the future, studies should address the capacity of invaders to displace neighboring species given a constant neighbor density and ecologically relevant environmental conditions. Moreover, more detailed work involving species-specific plant-plant interactions may be useful in predicting the impact on an invader on ecosystem processes.

3.5 Supplementary material

3.5.1 Tables

Table 7: ANOVA table of treatment effects on plant biomass. Role of the following fixed effects on *Microstegium* biomass (g), total plant biomass (g), and neighbor biomass (g) according to ANOVA results. Fixed effects include density treatment, neighbor treatment, and their interaction. Block was included as a random effect

Response	Fixed Effect	Sum of Squares	Mean Square	Num. df	Den. df	F value	p value
Microstegium biomass (g)	Density treatment	2726.77	908.92	3	97.99	31.71	<0.01
	Neighbor treatment	9324.76	4662.38	2	98	168.86	<0.01
	Interaction	544.28	90.71	6	97.99	3.3	0.01
Total plant biomass (g)	Density treatment	1720.03	430.01	4	125.02	3.11	0.02
	Neighbor treatment	100322.64	50161.32	2	125.02	382.45	<0.01
	Interaction	8413.98	1051.75	8	125.02	8.01	<0.01
Neighbor biomass (g)	Density treatment	9633.18	2408.29	4	81	15.37	<0.01
	Neighbor treatment	78076.1	78076.1	1	81	498.28	<0.01
	Interaction	383.14	95.78	4	81	0.61	0.66

Table 8: ANOVA table of soil responses to plant type. Role of plant type on soil measures (ammonium, nitrate, total inorganic N (μgN/G), ammonification, nitrification, mineralization (μgN/G×d), soil moisture (%)), and total plant biomass (g) according to ANOVA results. Plant type is a fixed effect with 4 levels: unplanted, *Microstegium*, *Panicum*, or *Sorghum* monoculture. Block was included as a random effect

Response	Fixed Effect	Sum of Squares	Mean Square	Num. df	Den. df	F value	p value
Ammonium (μgN/G)	Plant type	307.25	102.42	3	26.29	7.25	<0.01
Nitrate (μgN/G)	Plant type	23395.16	7798.39	3	26.28	43.59	<0.01
Total inorganic N (μgN/G)	Plant type	28742.7	9580.9	3	26.2	57.84	<0.01
Ammonification (μgN/G×d)	Plant type	7.03	2.34	3	26.72	1.56	0.22
Nitrification (μgN/G×d)	Plant type	12.16	4.05	3	26.25	0.56	0.64
Mineralization (μgN/G×d)	Plant type	34.86	11.62	3	26.35	1.12	0.36
Soil moisture (%)	Plant type	1313.1	437.7	3	35	12.63	<0.01
Total dry aboveground plant biomass (g)	Plant type	249789.2	83263.07	3	260.3	826.12	<0.01

Table 9: ANOVA table of soil responses to *Microstegium* biomass. Role of linear and quadratic *Microstegium* biomass fixed effects on soil measures according to ANOVA results. Block was included as a random effect

Response	Fixed Effect	Sum of Squares	Mean Square	Num. df	Den. df	F value	p value
Ammonium (μgN/G)	M.v. biomass	73.11	73.11	1	48.44	1.52	0.22
	(M.v. biomass)^2	15.93	15.93	1	49.42	0.57	0.45
Nitrate (μgN/G)	M.v. biomass	8691.99	8691.99	1	47.4	94.07	<0.01
	(M.v. biomass)^2	9737.14	9737.14	1	48.03	60.22	<0.01
Total inorganic N (μgN/G)	M.v. biomass	10333.93	10333.93	1	47.59	102.29	<0.01
	(M.v. biomass)^2	10640.1	10640.1	1	48.35	64.12	<0.01
Ammonification (μgN/G×d)	M.v. biomass	4.04	4.04	1	55	0.21	0.65
	(M.v. biomass)^2	0.02	0.02	1	55	0.02	0.9
Nitrification (μgN/G×d)	M.v. biomass	0.02	0.02	1	47.55	1.36	0.25
	(M.v. biomass)^2	10.59	10.59	1	48.25	1.55	0.22
Mineralization (μgN/G×d)	M.v. biomass	3.07	3.07	1	47.95	1.45	0.23
	(M.v. biomass)^2	10.63	10.63	1	48.8	1.15	0.29
Soil moisture (%)	M.v. biomass	197.97	197.97	1	48.24	2.17	0.15
	(M.v. biomass)^2	0	0	1	49.17	0	0.99

Table 10: ANOVA table of soil responses to density and neighbor treatments. Role of the following fixed effects on soil measures according to ANOVA results. Fixed effects include linear and quadratic *Microstegium* biomass (g) terms, neighbor treatment, and the interaction between *Microstegium* biomass and neighbor treatment. Block was included as a random effect

Response	Fixed Effect	Sum of Squares	Mean Square	Num df	Den. df	F value	p val
Ammonium (μgN/G)	M.v. biomass	26.25	26.25	1	104.3	0.63	0.43
	(M.v. biomass)^2	15.58	15.58	1	105.16	1.03	0.31
	Neighbor	187.26	93.63	2	104.65	3.35	0.04
	Interaction	31.05	15.53	2	105.19	0.59	0.56
Nitrate (μgN/G)	M.v. biomass	1614	1614	1	103.38	1.6	0.21
	(M.v. biomass)^2	260.86	260.86	1	103.63	3.94	0.05
	Neighbor	921.49	460.74	2	103.48	7.6	<0.01
	Interaction	505.06	252.53	2	103.64	2.89	0.06
Total inorganic N (μgN/G)	M.v. biomass	2086.28	2086.28	1	103.46	2.39	0.13
	(M.v. biomass)^2	393.48	393.48	1	103.77	5.37	0.02
	Neighbor	1686.22	843.11	2	103.59	10.67	<0.01
	Interaction	774.67	387.33	2	103.78	3.55	0.03
Ammonif. (μgN/G×d)	M.v. biomass	0.01	0.01	1	104.31	2.09	0.15
	(M.v. biomass)^2	0.1	0.1	1	105.21	2.93	0.09
	Neighbor	1.93	0.97	2	104.68	4.05	0.02
	Interaction	0.56	0.28	2	105.23	1.26	0.29
Nitrification (μgN/G×d)	M.v. biomass	4.52	4.52	1	103.86	5.76	0.02
	(M.v. biomass)^2	0.23	0.23	1	104.47	7.14	0.01
	Neighbor	49.17	24.58	2	104.11	5.19	0.01
	Interaction	28.39	14.19	2	104.48	2.95	0.06
Mineraliz. (μgN/G×d)	M.v. biomass	5.04	5.04	1	103.86	4.69	0.03
	(M.v. biomass)^2	0.03	0.03	1	104.46	5.69	0.02
	Neighbor	31.71	15.86	2	104.1	3.73	0.03
	Interaction	21.08	10.54	2	104.47	2.36	0.1
Soil moisture (%)	M.v. biomass	621.87	621.87	1	112	0.74	0.39
	(M.v. biomass)^2	650.28	650.28	1	112	0.01	0.94
	Neighbor	387.44	193.72	2	112	8.94	<0.01
	Interaction	127.04	63.52	2	112	2.34	0.1

Table 11: ANOVA table of soil responses to total plant biomass. Role of linear and quadratic total plant biomass terms on soil measures according to ANOVA results. Block was included as a random effect

Response	Fixed Effect	Sum of Squares	Mean Square	Num. df	Den. df	F value	p value
Ammonium (μgN/G)	Total biomass	300.7	300.7	1	108.99	0.45	0.5
	(Total biomass)^2	58.77	58.77	1	110.09	2.38	0.13
Nitrate (μgN/G)	Total biomass	2821.57	2821.57	1	107.5	5.43	0.02
	(Total biomass)^2	88.14	88.14	1	107.81	1.01	0.32
Total inorganic N (μgN/G)	Total biomass	4888.87	4888.87	1	107.58	3.25	0.07
	(Total biomass)^2	3.51	3.51	1	107.95	0.03	0.86
Ammonification (μgN/G×d)	Total biomass	1.83	1.83	1	109.19	0.41	0.52
	(Total biomass)^2	0.41	0.41	1	110.4	1.86	0.18
Nitrification (μgN/G×d)	Total biomass	13.62	13.62	1	108.54	0.26	0.61
	(Total biomass)^2	4.47	4.47	1	109.53	0.84	0.36
Mineralization (μgN/G×d)	Total biomass	5.22	5.22	1	108.41	0.15	0.7
	(Total biomass)^2	2.07	2.07	1	109.3	0.43	0.51
Soil moisture (%)	Total biomass	2311.49	2311.49	1	112.06	0.07	0.79
	(Total biomass)^2	107.92	107.92	1	114.41	5.35	0.02

4. Conditions that promote invader abundance can also limit its impact on soil nitrogen cycling

4.1 Introduction

Invasive plants can alter soil biogeochemistry in ways that impair ecosystem services, harm native species, and promote further invasions (Vitousek & Walker 1989; Ehrenfeld 2003; Bever *et al.* 2010). While the field of invasion biology has primarily worked to address how invasive species colonize and persist, understanding the impact of invasive species on ecosystem functioning has received increased attention (Davis *et al.* 2011; Vilà *et al.* 2011; Ricciardi *et al.* 2013; Hulme *et al.* 2013). Recent studies have demonstrated that invasive plants can have important biogeochemical consequences by altering litter inputs (Liao *et al.* 2008), the timing and location of nitrogen (N) released from decomposing litter (Laungani & Knops 2009), N outputs (Asner & Beatty 1996; Allison & Vitousek 2004; Hawkes *et al.* 2005; Laungani & Knops 2009), and soil carbon fluxes (Litton, Sandquist & Cordell 2008; Strickland *et al.* 2010; 2011). In cases where invader impacts have been quantified, however, there is a large amount of variability in the direction and magnitude of effects across the landscape (Hulme *et al.* 2013; Castro-Díez *et al.* 2014), which suggests that further investigation is necessary to resolve context-dependencies and improve our ability to predict the conditions in which one invasive species will have its most severe impacts. For individual invasive species and impacts, we must identify factors that mediate impact direction and magnitude and begin to understand how factors additively, interactively, directly, and indirectly contribute.

Historically, the magnitude of a species' effect on ecosystem processes has been predicted simply by its dominance: the mass-ratio hypothesis (Grime 2001; Mokany, Ash & Roxburgh 2008). This concept that a species' effect scales linearly with its abundance (Figure 15) is frequently used as a simplifying assumption (Parker *et al.* 1999; Ricciardi 2003), but rarely tested. Factors other than invader biomass can directly and indirectly mediate impact magnitude. Environmental conditions that control substrate availability and/or impose physical constraints on the target ecosystem process (e.g. soil organic matter, moisture, pH) can directly control the magnitude of an invader's impact (Figure 15). Environmental conditions can also mediate the shape of the relationship between invader abundance and impact magnitude or direction (Figure 15). These pathways may be equally important or even more important than the direct effect of invader abundance on impact sizes. There is theory and evidence to support the idea that both of these factors, species' abundance and environmental conditions, can mediate species' impacts on ecosystem processes (Grime 2001; Castro-Díez *et al.* 2014), but experimental studies that test the relative importance of these factors have been lacking. This knowledge gap has hindered the development of predictive models of how species gains and losses influence vital ecosystem services across a range of ecosystem types and environmental conditions (Pyšek *et al.* 2012; Hulme *et al.* 2013).

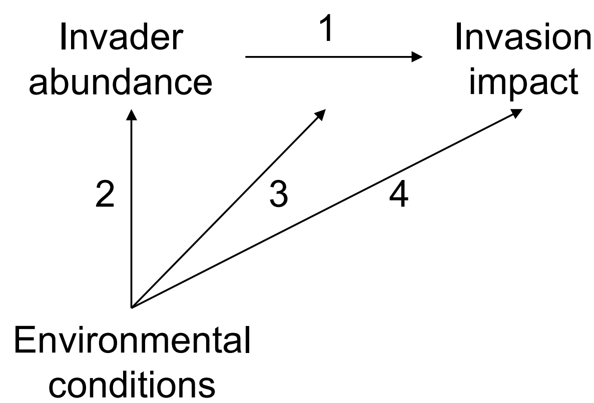

Figure 15: Environmental conditions can directly and indirectly mediate the magnitude and direction of invasion impacts on ecosystem processes. Invader abundance predicts the magnitude of an invasion impact (1), while environmental conditions can shape invader abundance (2), mediate the relationship between invader abundance and invasion impact (3), and directly modify the magnitude of an impact (4)

In addition to understanding the relative importance of the direct effects of these factors it is also important to consider associations among these factors that may indirectly mediate the magnitude that a species will alter an ecosystem process. The same environmental conditions that regulate invasion impacts may also play a part in regulating the species' abundance (Figure 15). For instance, very high soil moisture can inhibit nutrient mineralization by creating anoxic microsites. If a plant species with litter that promotes nutrient mineralization can only be highly abundant in habitats with very high soil moisture, then mineralization rates at sites with high soil moisture will be lower than expected because a direct negative effect modifies the indirect positive effect of soil moisture on mineralization. Synergies among environmental conditions and species' abundance can also occur if conditions that promote species abundance also promote the magnitude of its ecosystem impact.

Here, we use the invasive grass, *Microstegium vimineum*, to investigate pathways through which environmental conditions mediate invasion impacts on soil N cycling. *Microstegium* is one of the most highly studied invasive plant species, especially with regard to its impact on soil N pools and fluxes (Castro-Díez *et al.* 2014), and so can serve as a model system for in-depth study of factors that mediate variability in invader impacts on N cycling. A number of studies indicate that *Microstegium* alters soil properties and N dynamics, specifically elevating nitrate concentrations and net nitrification rates (Kourtev, Ehrenfeld & Huang 1998; Ehrenfeld *et al.* 2001; Kourtev *et al.* 2002). While *Microstegium* effects on nitrate and nitrification rates are typically positive, they are highly variable, as some studies report that invaded areas or treatments did not differ from reference areas (McGrath & Binkley 2009; Adams & Engelhardt 2009). Additionally, *Microstegium* increases decomposition rates, particularly promoting the loss of fast-cycling C pools (Strickland *et al.* 2010; 2011), although this pattern is not universal (Strickland *et al.* 2011). We hypothesized that increases in nitrate and net nitrification due to *Microstegium* presence would be greater at sites where *Microstegium* is more abundant, in line with the mass-ratio hypothesis, and that environmental factors that promote *Microstegium* abundance, *i.e.* soil moisture and light availability (Warren *et al.* 2010), would indirectly promote invader impacts. In addition, we hypothesized that soil organic matter and moisture would directly mediate invasion impacts on N pools and fluxes by controlling substrate availability and microbial activity and that these factors may interactively mediate invasion impacts by amplifying or suppressing invader effects on a per gram basis.

Our study takes place across a network of invasion fronts that vary in *Microstegium* abundance and environmental conditions. The objectives of our study are three-fold. Our first aim is to identify the direction and magnitude of mean impacts of *Microstegium vimineum* on soil N pools and fluxes across all study sites. Second, we will evaluate the additive and interactive effects of invader biomass and reference environmental conditions on site-level invasion impacts on nitrate, nitrification, and any soil N pool or flux that responded to invasion across sites. Third, we will determine the relative importance of direct and indirect pathways through which environmental reference conditions mediate soil N impacts.

4.2 Methods

4.2.1 Study design

In 2011, 18 sites were established at *Microstegium* invasion fronts within Duke Forest, Durham, NC. Duke Forest is 7000 acres of up to 70-year-old stands dominated by sweetgum, tulip poplar, oaks, hickories, and pines. Each site consisted of 2 plots, an invaded and reference plot, which were established as in (Warren *et al.* 2010). Invasive species other than *Microstegium* were sometimes present in the invaded and reference plots (e.g. *Lonicera japonica*).

4.2.2 Plot measurements

During peak growing season in 2012 and 2013, 3-0.25x0.25m quadrats per invaded and reference plot were sampled for vegetation and soil across the 18 sites. Harvested vegetation was sorted into *Microstegium* and non-*Microstegium* plant material and litter, dried, and weighed. Immediately after each vegetation harvest, 1 soil core was collected (0-15cm depth, 5cm diameter) from the center of each quadrat, split by depth (0-5cm, 5-15cm), and then pooled by plot. We split soils by depth to account for increases in soil organic matter with depth. Soils were sieved through a 2mm mesh within 48 h of field sampling. Soil inorganic N was extracted with 2M KCl (10:1) and measured with an auto-analyzer (Lachat, Hach Company, Loveland, CO). Net N mineralization rates (ammonification, nitrification, and mineralization) were estimated by incubating soils aerobically in the lab at 22° C for 12-14 days. Soil moisture was measured gravimetrically and the percentage of soil organic matter was determined by loss on ignition using a muffle furnace at 430° C. We determined the understory light availability of each plot during peak growing season by measuring photosynthetically active radiation above the vegetation canopy and at 1 meter above each quadrat, aggregated by plot, to calculate the percent of light reaching the understory (AccuPAR Linear PAR/LAI ceptometer; Decagon Devices, Pullman, Wash.).

4.2.3 Site measurements

In 2012, the tree canopy of each site was surveyed. Each tree greater than 3cm diameter at breast height (dbh) and within 10 meters of the invasion front point established in 2011 was identified to species and its dbh was measured. Based on this information and knowledge of tree species' mycorrhizal status, we calculated the number of trees per site, the tree basal area per site (m^2), and the percent of basal area accounted for by tree species that associate with arbuscular mycorrhizal fungi (hereafter, %AM basal area).

4.2.4 Reference conditions

To quantify reference plot conditions, we used an ordination approach because we had many more environmental variables relative to the number of sites. First, we ordinated the following variables using PCA: all soil measurements from 0-5cm and 5-15cm depths, understory plant biomass, litter biomass, understory light availability, number of trees, tree basal area, and %AM basal area. The first two principal components cumulatively explained 45.3% of variation the measured environmental variables across sites (Supplement: Figure 19). Principal component 1 (PC1; explains 28.2% of the variance) represented increasing soil moisture and organic matter and principal component 2 (PC2; explains 17.1% of the variance) represented variation in forest openness (Supplement: Figure 19, Supplement: Table 13). To aid in the interpretability of PC2 throughout the study, we multiplied PC2 scores by negative one so that increasing values consistently represent increases in forest openness, e.g. high light and low tree density.

4.2.5 Analyses

To determine whether the presence of *Microstegium* is associated with changes in soil N pools and fluxes, we tested for an association among invasion status in multivariate soil N pool and flux space using perMANOVA. To determine the role of invasion on individual soil N pools and fluxes in paired plots, we ran mixed effects models to predict the selected soil N pool or flux using invasion status as a fixed effect and site-year as a random effect.

We were also interested in whether site-level variation in invasion impacts on soil N pools and fluxes are a function of reference plot conditions, *Microstegium* biomass, and their interaction. General linear mixed effects models (GLMMs) were used to predict site-level invasion impacts on a soil N pool or flux using reference condition principal components, *Microstegium* biomass, and their interactions as fixed effects and year as a random effect. To avoid circularity in our analysis, we created a unique reference ordination to analyze each invasion impact measurement type. The reference ordination was unique for each analysis because it excluded reference variables associated with the impact measurement type. For example, the PC scores used to predict site-level invasion impacts on nitrate in 0-5cm come from an ordination that included all of the above-mentioned variables except reference plot nitrate concentrations in 0-5cm and 5-15cm depths. Across all reference condition ordinations, variables loaded on to PC1 and PC2 in qualitatively the same way (Supplement: Table 13). PC2 loadings differed in sign for ordinations without nitrate and without ammonification; so, as previously mentioned, we multiplied PC2 scores by negative one as needed to consistently represent this axis of environmental variation as increasing forest openness.

To determine whether reference site conditions predict *Microstegium* biomass at invasion fronts, we ran GLMMs to predict *Microstegium* biomass using PC1, PC2, and their interaction as fixed effects, and year as a random effect.

To determine the relative importance of direct and indirect pathways through which environmental conditions shape invasion impacts, we used a piecewise structural equation modeling approach (Shipley 2002), linking the GLMMs described above that explain invader biomass and site-level invasion impact in a causal network. We constructed the model set such that the first model uses PC1 and PC2 as fixed effects to predict *Microstegium* biomass and the second model uses *Microstegium* biomass, PC1, PC2, and their interactions as fixed effects. For both models, year is a random effect. Model fit was evaluated based on the Fisher's C statistic and a X^2 distribution significance test. Standardized and non-standardized path coefficients and the marginal and conditional R^2 values (Nakagawa & Schielzeth 2012) were extracted from model output (Lefcheck 2015). Only the nitrate, ammonification, nitrification, and mineralization impact measures collected from the 0-5cm soil depth were evaluated in this manner because previous GLMMs analyses demonstrated that one or more fixed effect (i.e., *Microstegium* biomass, PC1, PC1, and interactions) were not able to explain the other impact measures.

All analyses were conducted using R Statistical Software (R Development Core Team and Team 2012). For ordinations and perMANOVAs, we used base R and the vegan package (Oksanen *et al.* 2015). For all analyses with mixed effects models, we used the lme4 and lmerTest packages to fit the models, calculate degrees of freedom with Satterthwaite approximations, and run t-tests to evaluate whether fixed effect coefficients significantly differ from zero (Kuznetsova *et al.* 2014; Bates *et al.* 2015). Piecewise structural equation modeling was conducted using the piecewiseSEM package (Lefcheck 2015).

4.3 Results

4.3.1 Mean invasion impacts

Overall, our multivariate analysis of soil N pools and fluxes showed no difference between invaded and reference plots ($F_{1,55}$=1.75, p>.1). Accounting for site and year, invaded plots have faster ammonification rates in the 0-5cm soil depth (Figure 16, Supplement: Table 14, p<.05). Invaded plots also tend to have faster ammonification and mineralization rates at the 5-15cm depth although these effects were marginally significant (Figure 16, Supplement: Table 14, p=0.05 and p=0.06). For the following analyses of drivers of site-level invasion impacts, we did not investigate ammonium because we did not detect a mean effect of invasion across sites and there is no previous evidence that ammonium pools respond to the presence of *Microstegium*.

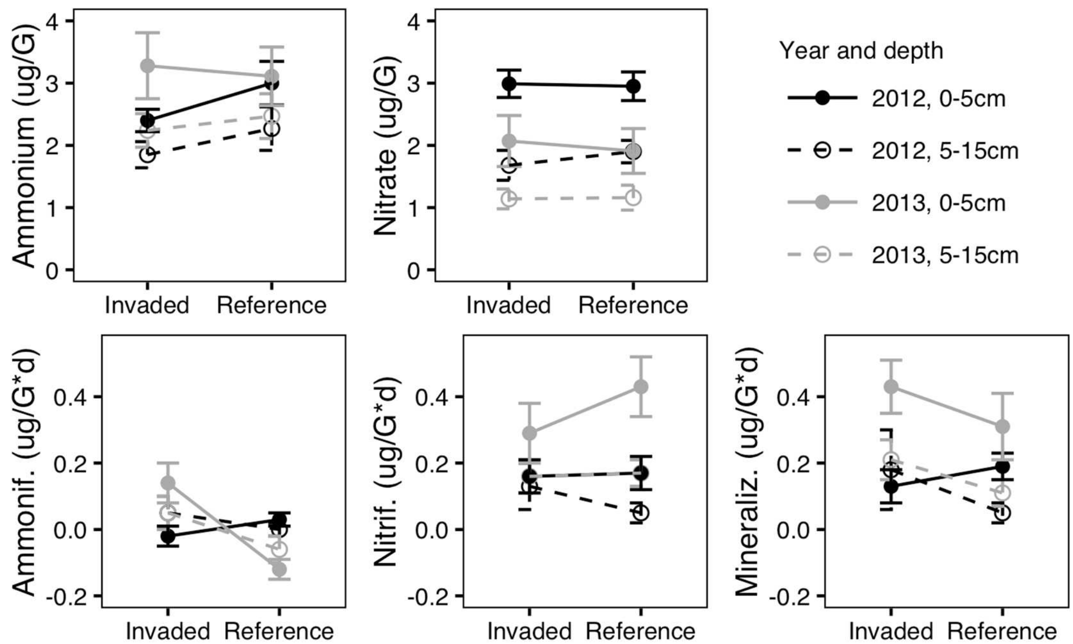

Figure 16: Aggregated across study sites, panels show variation, or lack thereof, in soil inorganic N pools and fluxes in invaded and reference plots by year and soil depth. Invaded plots tend to have higher ammonification rates and marginally higher mineralization rates (Supplement: Table 14; mean±SE

4.3.2 Drivers of site-level invasion impacts

At sites with low forest openness (PC2), more *Microstegium* biomass led to increases in soil nitrate in invaded areas, whereas the opposite trend is observed at sites with greater forest openness (Supplement: Figure 21, Supplement: Table 15, $p<.05$). Sites with high forest openness were sites with understory light availability >10%.

At a given site, more *Microstegium* biomass led to greater increases in net ammonification rates, especially at sites with high soil moisture and organic matter (Figure 17a, Supplement: Table 15). However, the impact of *Microstegium* presence of net ammonification rates diminished with increasing forest openness (PC2) in sites with high soil moisture and organic matter (PC1) (Figure 17b, Supplement: Table 15). As such, greater increases in net ammonification due to invasion were generally observed either (a) where there is low soil moisture and organic matter (Supplement: Figure 22), or (b) where there is high soil moisture and organic matter and (i) high *Microstegium* biomass or (ii) low forest openness (Figure 17). Sites with low soil moisture and organic matter ranged from 10-20% and 1-4%, whereas sites with high values ranged from 20-40% and 5-6%, respectively.

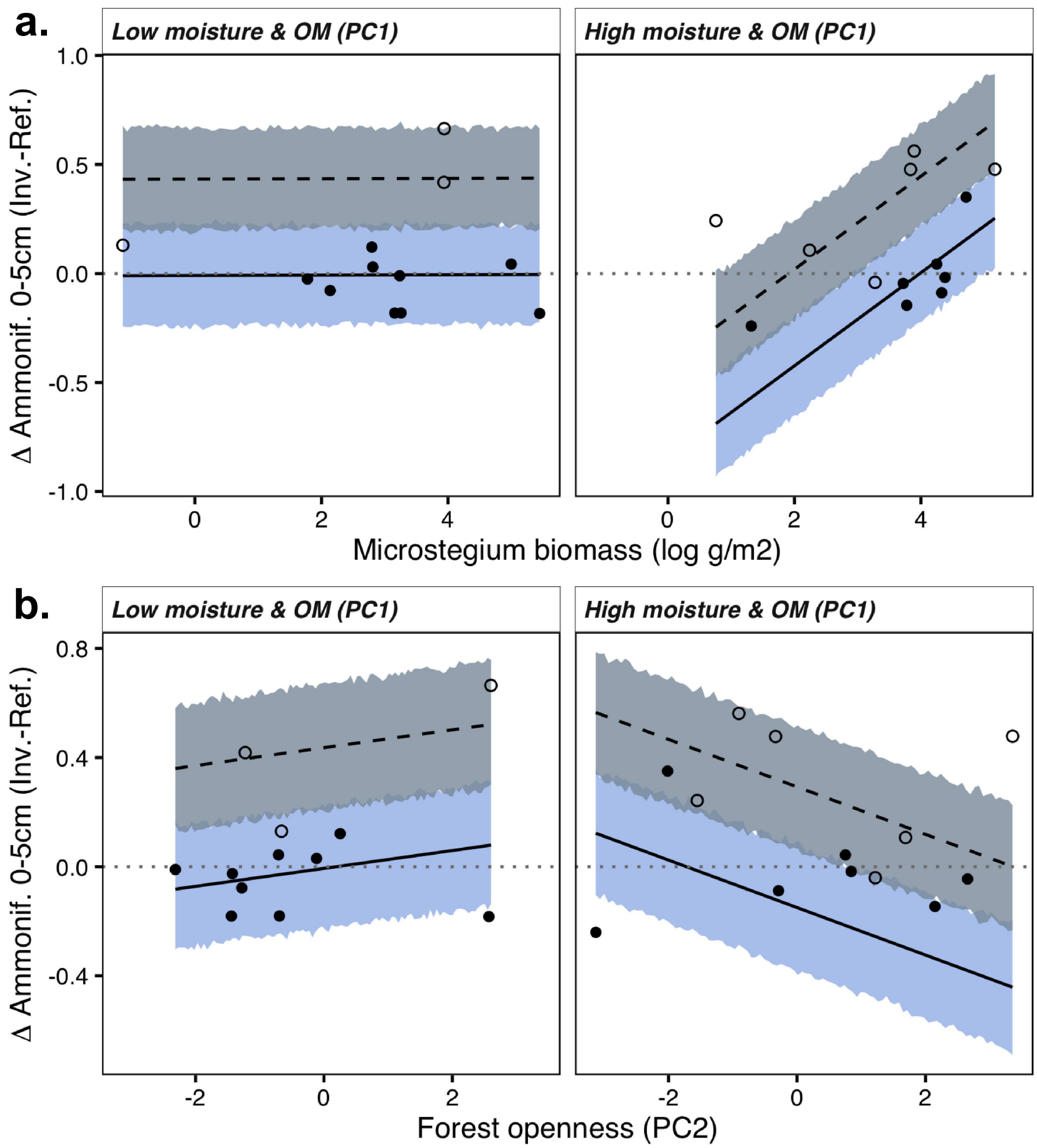

Figure 17: Site-level impacts of invasion on ammonification (Δ Ammonif.) at 0-5cm are explained in part by interactions among a) *Microstegium* biomass and reference soil moisture and organic matter (OM) conditions (PC1), and b) forest openness (PC2) and PC1. Observations in the left and right panels were split on the mean of PC1 to display these significant interactions (p<.05 for both). Low PC1 values represent sites with 10-20% moisture and 1-4% OM whereas high PC1 values represent sites with 20-40% moisture and 5-6% OM. Forest openness promotes *Microstegium* biomass (see Supplement: Figure 23), but in sites with high soil moisture and OM, these factors influence changes in ammonification in differing directions (right panels of a & b). Data from both years are shown (2012 = closed, 2013

= open circles) Model fits and 95% prediction intervals are shown, conditional on year (2012 = solid line and light blue, 2013 = dotted line and dark blue). See Supplement: Table 15 for full models results

Invasion impacts on mineralization at 0-5cm were shaped by a marginally significant negative interaction from among soil moisture and organic matter conditions (PC1) and *Microstegium* biomass (Supplement: Table 15). At sites with low soil moisture and organic matter (PC1), more *Microstegium* biomass led to increases in mineralization, whereas the opposite trend was observed at sites with high soil moisture and organic matter.

For the site-level invasion impacts on nitrification at 0-5cm, none of the fixed effects were statistically significant ((Supplement: Table 15). In addition, none of the fixed effects were statistically significant for site-level invasion impacts on any of the soil N pools or fluxes evaluated at 5-15cm ((Supplement: Table 15).

4.3.3 Relative importance of direct and indirect pathways

Each piecewise SEM model was adequately supported by the data and each model reflects the drivers of site-level invasion impacts that were reported in the previous section (Table 12; Figure 18; X^2=1.56, 0.76, 1.08; p>.05).

Table 12: Paths through which reference environmental conditions mediate site-level impacts of invasion on nitrate, ammonification, nitrification, or

mineralization at 0-5cm depth. Effect terms include soil moisture and organic matter (Soil moist. & OM, PC1), forest openness (PC2), and log-transformed *Microstegium* (*M.v.*) biomass, and their interactions. Effect sizes are calculated based on standardized path coefficients. Response values are calculated among paired invaded and reference plots such that Δ = Inv. – Ref

<table>
<tr><th>Response</th><th>Path</th><th>Effect</th><th>Effect size</th><th>Description</th></tr>
<tr><td>Δ Nitrate</td><td>Direct</td><td>Forest openness x M.v. biomass</td><td>-0.56</td><td>M.v. biomass promotes nitrate availability in closed forests, but reduces nitrate in open forests (Fig. S3)</td></tr>
<tr><td rowspan="7">Δ Ammonif.</td><td rowspan="5">Direct</td><td>M.v. biomass</td><td>0.65</td><td>M.v. biomass promotes impacts</td></tr>
<tr><td>Soil moist. & OM</td><td>-0.35</td><td>Soil moisture and OM diminish impacts (Fig. S4)</td></tr>
<tr><td>Soil moist. & OM x M.v. biomass</td><td>0.78</td><td>M.v. biomass promotes impacts only at high moisture and OM sites (Fig. 3a)</td></tr>
<tr><td>Soil moist. & OM x Forest openness</td><td>-0.52</td><td>Forest openness diminishes impacts in high moisture and OM sites (Fig. 3b)</td></tr>
<tr><td>SUBTOTAL</td><td>0.56</td><td></td></tr>
<tr><td>Indirect</td><td>Forest openness</td><td>0.31</td><td>Forest openness promotes M.v. biomass, which in turn promotes ammonification (Fig. S5).</td></tr>
<tr><td colspan="2">TOTAL</td><td>0.87</td><td></td></tr>
<tr><td>Δ Mineraliz.</td><td>Direct</td><td>Soil moist. & OM x M.v. biomass</td><td>-0.77</td><td>M.v. biomass tends to promote mineralization at low moisture and OM sites</td></tr>
</table>

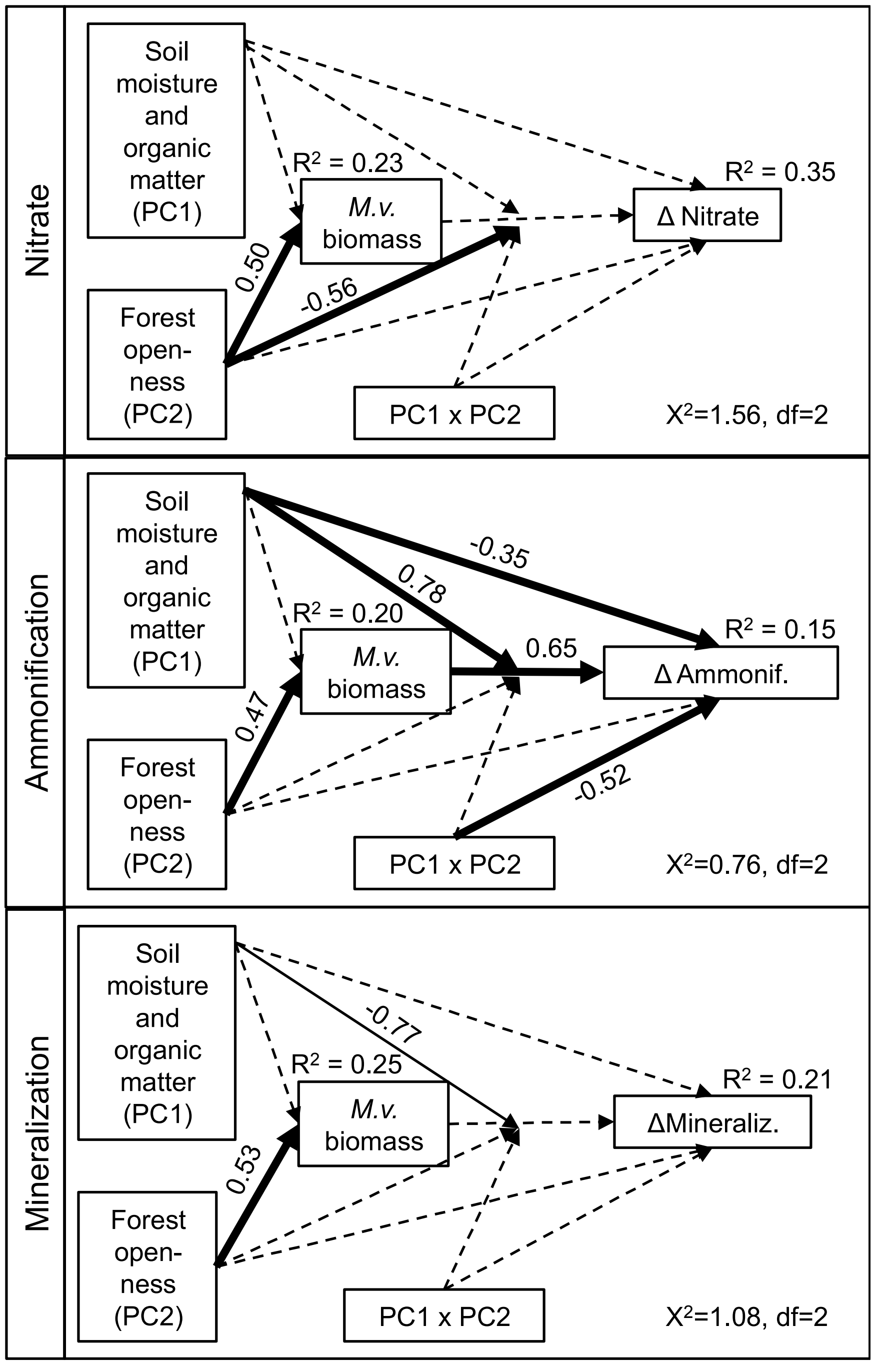

Figure 18: Environmental conditions (PC1 and PC2) can directly, indirectly, and interactively mediate site-level impacts of *Microstegium* (*M.v.*) invasion on nitrate, ammonification, and mineralization in 0-5cm depths. The magnitude and direction of these pathways differ by impact type. Path diagrams represent three piecewise structural equation models, each include year as a random effect. Arrows

that point to the path between *M.v.* biomass and impact (e.g. Δ Nitrate) illustrate an interaction term. Values on top of arrows indicate the standardized path coefficient. Solid bold arrows are significant (alpha=.05); the solid non-bold arrow is marginally significant (p=.06). Marginal R^2 values are provided

Microstegium biomass was greater at sites with more forest openness (PC2) (Supplement: Figure 23, p<.01), but did not vary based on the site's soil moisture and organic matter (PC1) nor the interaction between PC1 and PC2. The impact of invasion on net ammonification was the only impact type for which *Microstegium* biomass was an important driver of impact magnitude or direction. As such, forest openness had an indirect positive effect on the impact of invasion on ammonification (Table 12, Figure 18). In total, however, four causal pathways drove invasion impacts on ammonification and this indirect path had the smallest absolute effect size. The three other pathways, listed in decreasing absolute effect size are i) a positive interaction effect from among soil moisture and organic matter conditions (PC1) and *Microstegium* biomass, ii) a negative interaction effect from among PC1 and forest openness (PC2), iii) a direct negative effect from PC1 (Table 12, Figure 18).

4.4 Discussion

Through an in-depth study of how environment mediates an invasive species' impacts on soil N cycling, we see that environmental factors can (i) be highly influential, (ii) mediate impacts through multiple pathways, and (iii) have very different effects on related aspects of soil N cycling. These results, derived from one invasive species, demonstrate the high variability in controls and responses possible within a single system and indicate that future research efforts are necessary to contextualize how environmental factors mediate the impacts of invasion on soil processes more broadly. *Microstegium* is widespread invasive in the eastern U.S. (Warren *et al.* 2010) and predicting its influences on soil biogeochemistry will be necessary for managers working to maintain forest ecosystem health. Furthermore, it is a canonical invasive species (Castro-Díez *et al.* 2014), and these results indicate that how we understand invasive impacts will need to be much more context specific than previous research has achieved. In particular, environmental conditions can alter invasive impacts directly, indirectly, or interactively, and as such can prevent detection of global effects even when strong site-level impacts are present.

Environmental factors can control even the direction of the relationship between an invader's abundance and its impact. In the case of *Microstegium* impacts on nitrate, we found that greater quantities of invader biomass were tied to larger impacts at closed forest sites, e.g. low understory light availability and a high density of trees; whereas, the opposite pattern was detected in sites with greater forest openness. A hypothesis put forward by Ehrenfeld *et al.* (2001) suggests that *Microstegium* indirectly increases soil nitrate concentrations in invaded relative to reference areas by displacing plant species with higher nitrogen demands. Our data support this hypothesis because we only found a positive effect of *Microstegium* biomass on nitrate impacts at sites with high tree densities, where presumably nitrogen demand is high. We see the opposite trend in sites with few trees which is consistent with the idea that the addition of *Microstegium* biomass constitutes a net gain N demand at sites with fewer trees and relatively low plant N demand prior to invasion. Given that our study sites spanned areas of high and low forest openness, we did not find that areas invaded by *Microstegium* have higher nitrate concentrations on average, unlike a number of previous studies (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Adams & Engelhardt 2009; DeMeester & deB Richter 2010; Lee *et al.* 2012). Previous studies may have produced this signal in nitrate because they were conducted in closed-canopy forest sites with low light conditions and many trees. The majority of the seminal work that linked *Microstegium* with changes in N cycling was conducted in mature hardwood forests (Kourtev *et al.* 1998; 1999; Ehrenfeld *et al.* 2001), as *Microstegium* can invade and persist in the forest understory at low light levels (Horton & Neufeld 1998). Interactive effects of environmental factors and invader biomass on invader impacts, especially negative interactions, can lead to net zero mean effect sizes at larger scales and make it easy to overlook site-level impacts. This finding highlights that the nature of invasive species' impacts on ecosystems processes can be – to a large extent - a function of the local environment. In light of the potentially strong influence of environment, we may need to re-conceptualize invasion impacts as a function of the exotic species' characteristics and the invaded area's characteristics as recent reviews have suggested (Hulme *et al.* 2013; Castro-Díez *et al.* 2014), moving away from the traditional emphasis on solely exotic species' characteristics as drivers of impact direction and magnitude. This shift will be necessary to anticipate and remediate invader impacts across a heterogeneous landscape.

Environmental conditions can mediate invader impacts simultaneously via the three proposed pathways: indirectly, interactively, and directly (Figure 15). In the case of *Microstegium*'s impacts on ammonification, we identified 5 pathways through which impacts were mediated by two composite environmental factors, soil moisture and organic matter conditions and forest openness (Table 12). Cumulatively, these effects resulted in 2.5x higher net ammonification rates in invaded areas relative to reference areas on average across sites at 0-5cm. Although a number of studies have been published on the topic of *Microstegium*'s impacts on soil N cycling (Kourtev *et al.* 1998; 1999; Ehrenfeld *et al.* 2001; Adams & Engelhardt 2009; DeMeester & deB Richter 2010; Fraterrigo *et al.* 2011; Lee *et al.* 2012), this is the first time that an association between *Microstegium* and ammonification has been reported. This finding suggests that *Microstegium* presence causes an increase in ammonification. However, it should be noted that the observational nature of our study design does not allow us to rule out the idea that *Microstegium* presence in invaded plots is a consequence, rather than a cause, of high net ammonification rates. Our study design of paired invasion front plots made every effort to minimize the chance that differences among the paired plots pre-dated the presence of the invasion. Moreover, there is evidence that *Microstegium* reduces the litter layer (Kourtev *et al.* 1999), increases decomposition (Ehrenfeld *et al.* 2001), and promotes the mineralization of soil carbon (Strickland *et al.* 2010) so increases in ammonification due to *Microstegium* invasion is not unlikely.

Because environmental conditions can mediate invader impacts simultaneously via multiple pathways, this can lead to situations in which the directional influence of one pathway is at odds with another such that the same environmental factor that promotes invader abundance can also limit its impacts at a given site. In this study, we found that forest openness can both promote invader abundance and limit its impact on ammonification at sites with high soil moisture and organic matter. This set of relationships makes it difficult to predict which sites with high soil moisture and organic matter will have the largest ammonification impacts without first knowing the relative importance of each pathway. In other words, will invasion have larger effects on ammonification where the invader is more abundant and forest openness is high or where the invader is less abundant and forest openness is low? Since we know from our path analysis that the direct impact of *Microstegium* biomass on ammonification is strong and that soil moisture and organic matter conditions have a strong positive interaction with *Microstegium* biomass, promoting its impacts, we can safely conclude that *Microstegium* impacts on ammonification are likely be greatest where the invader is most abundant, regardless of environmental conditions.

Overall, our results demonstrate that there is a lot of variability in how the same environmental factors can shape invader impacts on related components of soil N cycling. For example, an interaction effect among soil moisture and organic matter conditions and *Microstegium* biomass were found to mediate invasion impacts on both net ammonification and mineralization, but their signs are opposite (Table 12). In addition, the two composite environmental factors were entirely unsuccessful at explaining variability in invader impacts on nitrification. Previous studies report that areas invaded by *Microstegium* have higher net nitrification rates, yet our findings do not support this. Moreover, unlike *Microstegium* impacts on nitrate, we did not detect a negative interaction effect among invader biomass and an environmental factor that could explain the absence of a global mean effect.

One explanation for why nitrification did not respond to *Microstegium* invasion in a way similar to previous studies (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Lee *et al.* 2012) is that soil pH is relatively low across all our study sites. Many of our sites have acidic soils and are in forested areas in the presence of pine trees or former pine plantations, whereas previous studies' sites did not occur on this type of land use. It well known that the activity of nitrifying bacteria is inhibited in low pH soil conditions and this may have contributed to the absence of an effect of *Microstegium* presence on nitrification on average. Another explanation for this discrepancy is that other studies may have sampled soil with more dense *Microstegium* abundance. In an effort to capture invasion fronts, we sampled at the edge of invasions where the *Microstegium* density was moderate-to-low relative to the center of the invaded areas. Less than half of the invaded plots included in our study had more than 50% Microstegium cover. It should be noted, however, that significant effects of *Microstegium* presence on nitrate and nitrification have been reported from sites with densities as low as 1-20% cover (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001).

Invasive plant impacts on soil nitrogen cycling are context dependent and can be highly variable. Our study provides evidence that consideration of environmental context is critical for understanding where, or under what conditions, the impacts of an invasive plant species will be most severe. We show that environmental factors can be so influential that they control even the direction of the relationship between an invader's abundance and its impact. Moreover, environmental conditions can mediate invader impacts simultaneously via the three proposed pathways: indirectly, interactively, and directly (Figure 15). This can lead to situations in which the same environmental factor that promotes invader abundance can also limit its impacts at a given site. Last, environmental factors mediate different, yet biogeochemically-linked, invader impacts on soil N cycling through different pathways and to varying degrees. This information regarding *Microstegium*'s impacts on soil N cycling will be useful for land managers who would like to manage this highly invasive species for its impacts on soil nitrogen. More broadly, our results serve to emphasize that invasive species impacts do not happen in a vacuum and that further study is needed to understand how, for many species and systems, environmental factors mediate the ecosystem impacts of invasion.

4.5 Supplemental material

4.5.1 Tables

Table 13: PC variable contributions for reference plot ordinations with all (Full) and without (w/o) a subset of variables. Factors that contribute > 10% are shown. Full model includes ammonium, nitrate, ammonification, nitrification, mineralization, soil moisture, organic matter, and pH from 0-5cm and 5-15cm, plant biomass, light avail., litter biomass, and tree density, basal area, and %AM basal area

PC	Model	Variable	Contribution (%)	Loading
PC1	Full	Soil moisture (0-5cm)	14.12	0.38
		Soil moisture (5-15cm)	11.51	0.34
		Nitrif. (0-5cm)	11.29	0.34
		Organic matter (0-5cm)	10.53	0.32
	w/o Nitrate	Soil moisture (0-5cm)	14.47	0.38
		Nitrif. (0-5cm)	12.89	0.36
		Soil moisture (5-15cm)	12.09	0.35
		Organic matter (0-5cm)	10.84	0.33
	w/o Ammonif.	Soil moisture (0-5cm)	14.73	0.38
		Soil moisture (5-15cm)	12.28	0.35
		Organic matter (0-5cm)	12.2	0.35
		Nitrif. (0-5cm)	11.45	0.34
	w/o Nitrif.	Soil moisture (0-5cm)	15.76	0.4
		Soil moisture (5-15cm)	12.64	0.36
		Organic matter (0-5cm)	11.2	0.33
	w/o Mineraliz.	Soil moisture (0-5cm)	15.07	0.39
		Soil moisture (5-15cm)	12.07	0.35
		Nitrif. (0-5cm)	10.7	0.33
		Organic matter (0-5cm)	10.5	0.32
PC2	Full	Tree density	17.91	0.42
		Light availability	17.29	-0.42
		%AM	16.25	0.4
		Litter biomass	15.43	0.39
	w/o Nitrate	Tree density	21.7	-0.47
		Light availability	19.82	0.45
		%AM	16.96	-0.41
		Litter biomass	13.4	-0.37
	w/o Ammonif.	%AM	20.82	-0.46
		Tree density	17.59	-0.42
		Light availability	15.76	0.4
		Soil pH (0-5cm)	12.71	0.36
	w/o Nitrif.	Tree density	15.73	0.4
		Light availability	15.16	-0.39
		Litter biomass	14.56	0.38
		%AM	11.93	0.35
	w/o Mineraliz.	Tree density	17.85	0.42
		Light availability	17.28	-0.42
		%AM	17.12	0.41
		Litter biomass	15.81	0.4

Table 14: Summary of the effects of *Microstegium* invasion on soil N pools and fluxes. Plot type, invaded or reference, is the fixed effect for which estimates, standard error, degrees of freedom and p-values are shown. Year is a random effect. Fixed effect coefficients that significantly differ from zero are bold and gray-highlighted (alpha=.05), marginally significant coefficients are gray-highlighted (alpha=.1)

Response variable	**0-5cm**				**5-15cm**			
	Est	**SE**	**df**	**p-val**	**Est**	**SE**	**df**	**p-val**
Ammonium	0.01	0.29	25.41	0.98	-0.16	0.19	23.90	0.41
Nitrate	0.19	0.18	25.54	0.32	-0.08	0.11	25.72	0.51
Ammonif.	**0.12**	**0.04**	**55.00**	**0.01**	0.08	0.04	21.63	0.05
Nitrif.	-0.06	0.08	30.00	0.42	0.03	0.03	28.31	0.34
Mineraliz.	0.06	0.08	55.00	0.47	0.11	0.06	28.59	0.06

Table 15: Summary of mixed effects models used to predict site-level invasion impacts on nitrate, ammonification, nitrification, or mineralization at 0-5cm or 5-15cm depth. Fixed effects include soil moisture and organic matter (PC1), forest openness (PC2), log-transformed *Microstegium* biomass (Mv), and interactions, with year as a random effect. Fixed effect coefficients that significantly differ from zero are bold and gray-highlighted (alpha=.05), marginally significant coefficients are gray-highlighted (alpha=.1)

Response variable	Term	0-5cm				5-15cm			
		Est	SE	df	p-val	Est	SE	df	p-val
Δ Nitrate (Inv.-Ref.)	(Intercept)	0.98	0.69	16.00	0.18	0.15	0.72	16.00	0.84
	PC1	-0.57	0.43	16.00	0.20	0.06	0.45	16.00	0.90
	PC2	0.87	0.44	16.00	0.07	0.17	0.46	16.00	0.72
	Mv	-0.09	0.18	16.00	0.63	-0.04	0.19	16.00	0.85
	PC1:PC2	-0.35	0.26	16.00	0.20	-0.03	0.27	16.00	0.90
	PC1:Mv	0.15	0.11	16.00	0.18	-0.02	0.11	16.00	0.90
	PC2:Mv	**-0.23**	**0.10**	**16.00**	**0.04**	-0.08	0.11	16.00	0.47
	PC1:PC2:Mv	0.05	0.06	16.00	0.42	0.00	0.06	16.00	0.99
Δ Ammonif. (Inv.-Ref.)	(Intercept)	-0.23	0.24	1.38	0.49	-0.04	0.13	13.00	0.75
	PC1	**-0.22**	**0.06**	**16.01**	**<0.01**	0.01	0.08	13.00	0.94
	PC2	-0.03	0.06	16.04	0.57	-0.02	0.09	13.00	0.81
	Mv	**0.11**	**0.03**	**16.09**	**<0.01**	0.01	0.04	13.00	0.75
	PC1:PC2	**-0.07**	**0.03**	**16.00**	**0.04**	0.00	0.06	13.00	1.00
	PC1:Mv	**0.06**	**0.02**	**16.00**	**<0.01**	0.00	0.02	13.00	0.91
	PC2:Mv	0.00	0.01	16.02	0.91	0.02	0.02	13.00	0.37
	PC1:PC2:Mv	0.01	0.01	16.00	0.11	0.00	0.01	13.00	0.79
Δ Nitrif. (Inv.-Ref.)	(Intercept)	0.23	0.29	17.00	0.43	0.15	0.15	5.68	0.35
	PC1	0.25	0.18	17.00	0.19	0.04	0.07	14.06	0.62
	PC2	0.12	0.20	17.00	0.57	0.10	0.09	14.35	0.27
	Mv	-0.07	0.07	17.00	0.33	-0.03	0.03	14.62	0.36
	PC1:PC2	0.05	0.09	17.00	0.59	0.01	0.04	14.03	0.73
	PC1:Mv	-0.08	0.05	17.00	0.10	-0.01	0.02	14.04	0.63
	PC2:Mv	-0.02	0.05	17.00	0.67	-0.02	0.02	14.16	0.37
	PC1:PC2:Mv	-0.01	0.02	17.00	0.83	-0.01	0.01	14.06	0.45
Δ Mineraliz. (Inv.-Ref.)	(Intercept)	0.31	0.25	5.89	0.27	0.22	0.22	5.42	0.36
	PC1	0.25	0.14	15.27	0.09	0.10	0.12	15.24	0.43
	PC2	0.14	0.14	15.69	0.33	0.19	0.12	15.63	0.15
	Mv	-0.05	0.06	15.94	0.44	-0.05	0.05	15.90	0.34
	PC1:PC2	0.01	0.07	15.23	0.89	-0.04	0.06	15.20	0.46
	PC1:Mv	-0.07	0.04	15.16	0.06	-0.02	0.03	15.14	0.51
	PC2:Mv	-0.02	0.03	15.35	0.54	-0.02	0.03	15.31	0.44
	PC1:PC2:Mv	0.01	0.02	15.22	0.60	0.01	0.01	15.20	0.50

4.5.2 Figures

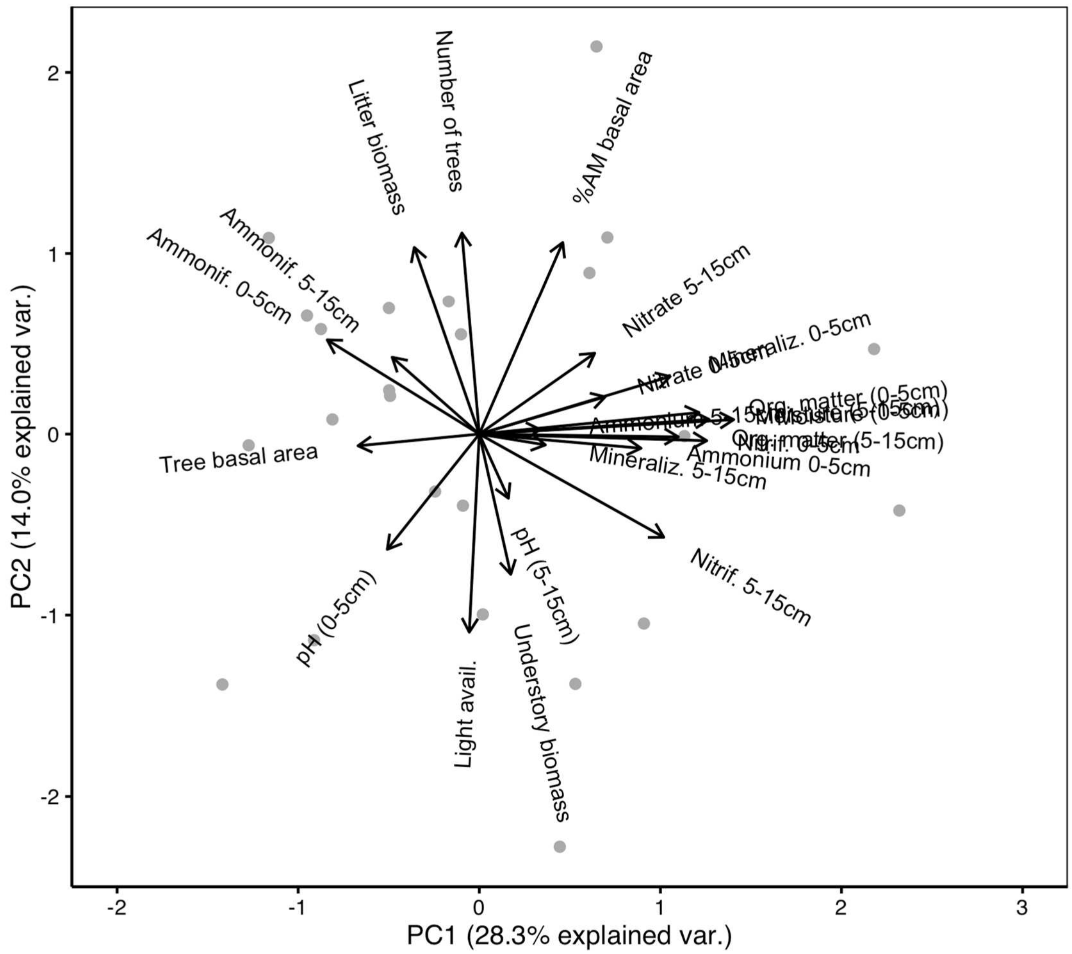

Figure 19: Principle components analysis of reference environmental characteristics across sites. Points represent reference plots in environmental parameter space; arrows indicate the direction and magnitude that each environmental parameter loads onto PC1 and PC2, which together explain 42.3% of variation. PC1 represents increases in soil moisture and soil organic matter, whereas PC2 represents increases in forest density. For all statistical analyses using PC2 scores, values were multiplied by -1 to represent 'forest openness' and ease interpretability. See Supplement: Table 13 for variable contributions and loadings

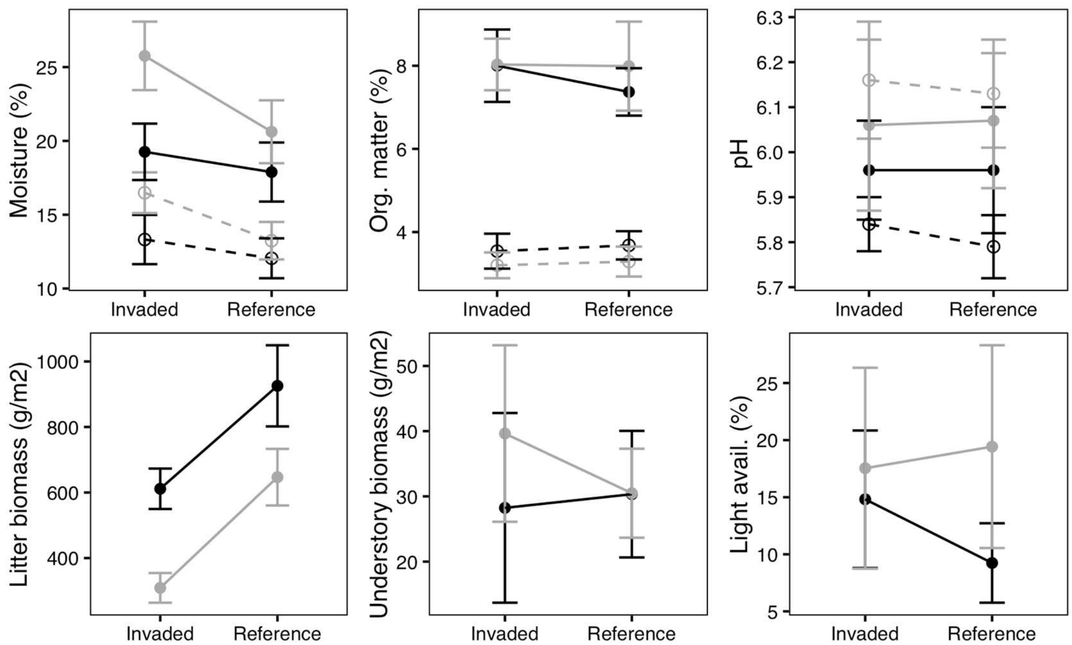

Figure 20: Effects of invasion on soil properties, litter and understory plant biomass, and understory light availability are shown. Soil properties include soil moisture, organic matter, and pH. Invaded plots tend to have higher soil moisture (0-5cm p<.05, 5-15 p<.01), and lower litter biomass (p<.001). Soil data from 2012 (black) and 2013 (gray) is presented at 0-5cm (solid line) and 5-15cm (short dashed line) depths; non-soil data is shown with solid lines; mean±SE

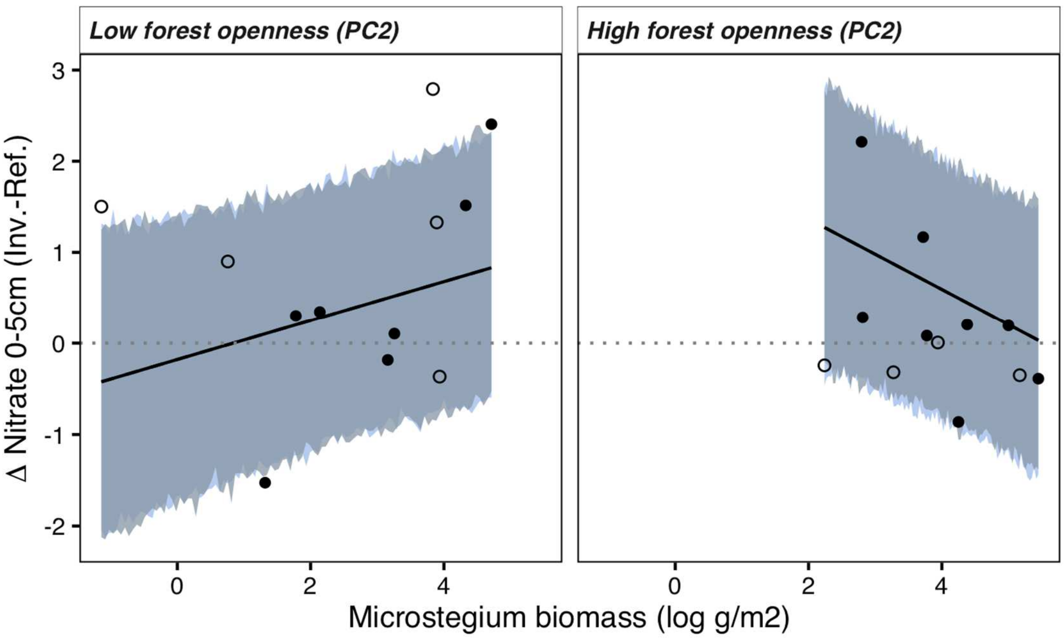

Figure 21: Site-level impacts of invasion on nitrate (Δ Nitrate) at 0-5cm are explained by a negative interaction among *Microstegium* biomass and forest openness (PC2). Panels show observations with low and high PC2 values, split on the mean, to display the interactive effect. Impact values increase with *Microstegium* biomass at sites with low forest openness (<5% understory light availability), whereas the opposite trend is observed in more open forest sites (>10% light) (Mv x PC2 interaction p<.05). Data from both years are shown (2012 = closed, 2013 = open circles) Model fits and 95% prediction intervals are shown, conditional on year (2012 = solid line and light blue, 2013 = dotted line and dark blue). See Supplement: Table 15 for full models results

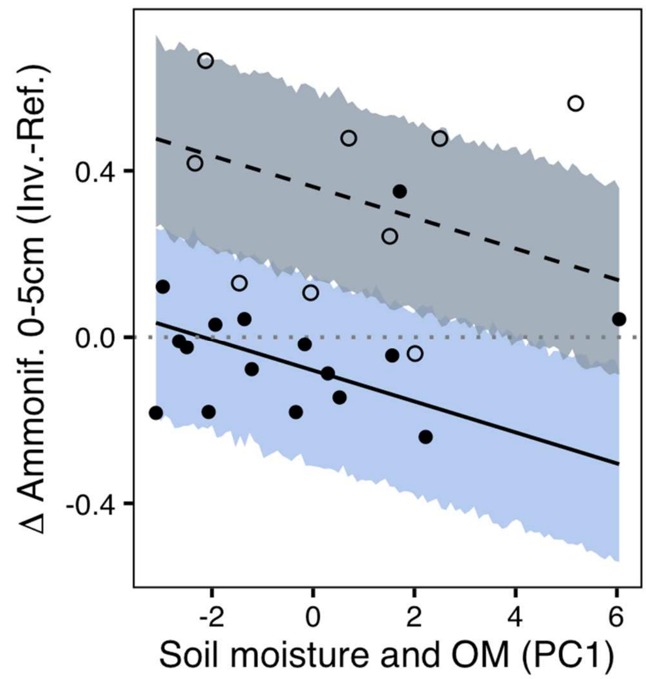

Figure 22: Soil moisture and organic matter (PC1) diminish site-level impacts of invasion on ammonification (Δ Ammonif.) at 0-5cm (p<.05). Data from both years are shown (2012 = closed, 2013 = open circles) Model fits and 95% prediction intervals are shown, conditional on year (2012 = solid line and light blue, 2013 = dotted line and dark blue). See Supplement: Table 15 for full models results

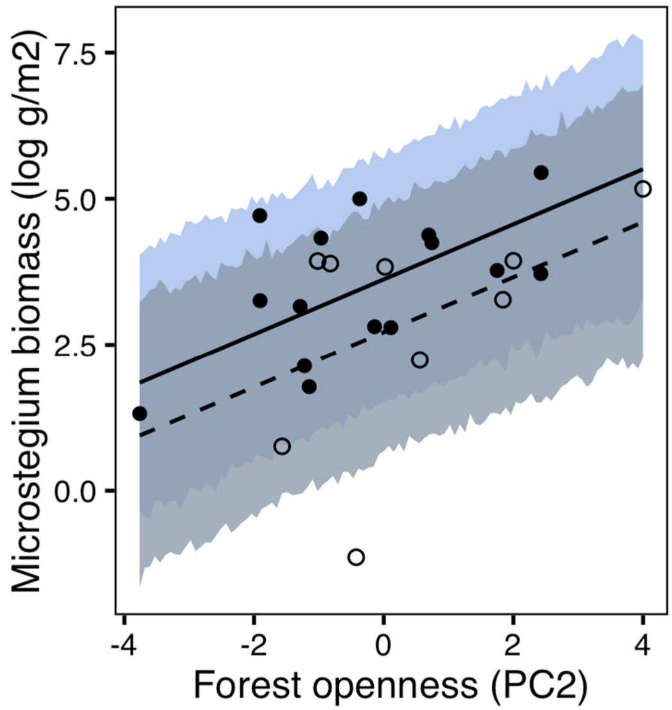

Figure 23: Forest openness (PC2) promotes *Microstegium* biomass. Full model includes PC1, PC2 and their interaction as fixed effects and year as a random effect; PC2 was the only significant fixed effect (p<.01). Data from both years are shown (2012 = closed, 2013 = open circles) Model fits and 95% prediction intervals are shown, conditional on year (2012 = solid line and light blue, 2013 = dotted line and dark blue).

5. Conclusions

Invasive species and their ecological consequences will continue to be an ecological issue that we face with increasing globalization. This dissertation sheds light on factors that mediate the magnitude and direction of that invasion impacts on one type of ecosystem process, soil N cycling, with the hope that we will one day be able to predict, anticipate, and mediate the consequences of invasive species and manage for healthy ecosystems.

In line with previous reviews on this topic, we found that, globally, invasions increase soil N pools and fluxes and that impact magnitudes and directions can be highly variable from species-to-species and ecosystem-to-ecosystem. Indeed, our model-invasive species, *Microstegium vimineum*, exhibited a significant amount of variability in the magnitude and direction of its impacts on N cycling across naturally invaded areas within Durham, North Carolina.

With regard to factors that mediate invasion impacts among species and ecosystems, previous literature focused on the role of the invasive species' N-fixing status and ecosystem type (Liao *et al.* 2008; Vilà *et al.* 2011; Castro-Díez *et al.* 2014). This dissertation made a number of important advances. First, we found that invasive species' leaf and litter traits, but especially trait novelty, shape invasion impacts on N cycling (Chapter 2). Plant tissue chemistry is sometimes invoked as a driver of invasion impacts on soil N cycling, especially for invasions that involve a non-N-fixing invasive species (Scharfy *et al.* 2011), but the analyses presented here on the relationship between trait values and impact magnitude and direction is the first global synthesis of its kind. Next, we presented evidence that the identity of neighboring species mediates the impact of our model invasive species, *Microstegium*, on soil N pools and fluxes (Chapter 3). This finding should serve as a cautionary tale against using solely a linear factor of invader density to estimate its impact on soil properties, particularly across areas where neighbor abundance or identity varies. Last, we presented evidence that environmental conditions mediate *Microstegium* impacts on soil N cycling through direct, indirect, and interactive mechanisms, via controls on invader biomass (Chapter 4). These findings emphasize the need to incorporate interactive and indirect effects of environmental context into our estimates of species effects on soil processes.

In combination, the studies presented in these chapters highlight three important points as we continue to study factors that mediate invasion impacts on ecosystem processes: (1) plant community functional traits mediate changes in ecosystem processes due to invasion, (2) a single driver can shape invasion impacts through multiple pathways, leading to unexpected results, and (3) we need a better conceptual synthesis for understanding the relative importance of invader abundance and trait dissimilarity on invader impacts.

Regarding plant functional traits, theory underpinning this concept has been present in the literature for some time (Suding, Goldberg & Hartman 2003; Suding *et al.*

2008), but a synthesis of the implications of altered community trait values in the context of invasion impacts on soil N has been lacking (Drenovsky *et al.* 2012). Some of the cause for this delay has been because trait measurements are often collected, if at all, on only a small subset of species present in invaded and reference plots. As such, we were only able to construct the analyses presented in Chapter 2 by supplementing our meta-analysis dataset with trait database data. Going forward, greater inclusion of trait measurements in invasion studies will greatly improve our ability to draw inference about predicted impacts. Moreover, we suggest focusing on calculations of the functional dissimilarity between invaded and reference communities. Sometimes, as we found in the greenhouse study on *Microstegium*'s impacts on soil N cycling (Chapter 3), the strong actors are the neighboring species instead of the focal invasive species. Measures of functional dissimilarity among invaded and reference plant communities are able to account for direct effects of invasive species traits, as well as the indirect and/or interactive effects of neighboring species' traits. The next steps in understanding the impact of invasion via plant functional trait traits will involve progress identifying the relative importance of different functional traits and potential interaction effects among those traits.

This leads us to our next point of discussion, that a single factor can shape invasion impacts through multiple pathways. In the fourth chapter, we observed that forest openness had direct, indirect, and interactive effects on the impact of *Microstegium* invasion on ammonification rates. It was only through knowledge of the relative importance of those pathways that we were able to conclude that invasion promotes ammonification most in regions where invader abundance is high and where, counter-intuitively, environmental conditions are also limiting changes in ammonification due to *Microstegium* presence. When we investigate factors that mediate invasion impacts, we are really investigating two moving parts: an invasive species' abundance and the state of an ecosystem process. As such, we must be aware that factors that mediate one of these components may also mediate the other, but not necessarily in the same direction or magnitude. Researchers should consider using this systems-based approach as they move forward in analyzing factors that mediate invasion impacts on ecosystem processes.

Throughout this dissertation, we have been implicitly discussing factors that mediate invasion impacts on soil N cycling within the framework of two, non-mutually exclusive hypotheses: the mass ratio (Grime 2001) and novel-traits (Callaway *et al.* 2008; Scharfy *et al.* 2011) hypotheses. The mass ratio hypothesis suggests that a species' abundance controls its ecosystem effects whereas the novel traits hypothesis emphasizes the role of species' novelty in determining its impacts. The findings that we present in Chapters 2 & 3 lend empirical support to both hypotheses, but in future work we need to understand the relative importance of these forces in shaping the direction and magnitude of invasion impacts on ecosystem processes. Moreover, by applying what we have learned from Chapter 4 about the significance of using a systems-based approach, we can see that the relationship between invader abundance and novelty may be critical to predicting invasion impacts. An invasive species may be abundant in its new range because it is functionally dissimilar, occupying functional space that is unused by resident species ("niche-filling species"; (Ordonez 2014). Alternatively, an invasive species may be successful by matching resident species' niches and out-competing them ("niche-matching" species; (Ordonez 2014). For one invasive species, these alternate hypotheses generate predictions about where its impacts on ecosystem processes will be greatest (Figure 1a). In particular, negative co-variation among invader abundance and novelty leads to uncertainty in where in the ecosystem impacts will be greatest (Figure 1b).

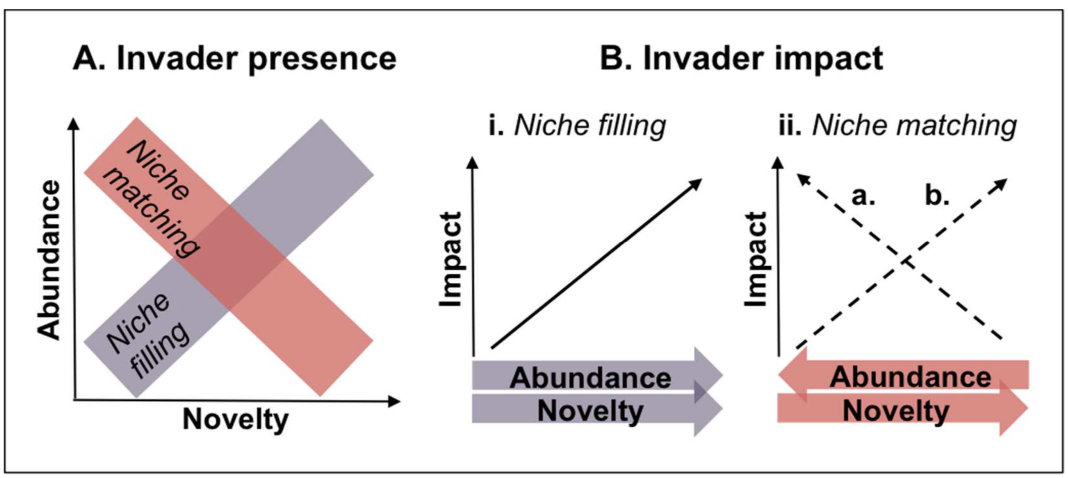

Figure 24: An invasive species can have invasive success (i.e. high abundance) by either *filling* or *matching* functional characteristics of resident species [19]. The two mechanisms can lead to different relationships between an invasive species' abundance and novelty (A). The ecosystem impact of niche-filling invaders will be greatest where the species is most abundant and novel (B.i.). It is unknown whether

the ecosystem impacts of niche-matching invaders will be greatest where the species is most abundant (B.ii.a.), or most novel (B.ii.b.)

Are impacts greatest where the invasive species is most abundant and least novel, in line with the mass-ratio hypothesis? Or where the species is least abundant and most functionally dissimilar, in line with the novel-traits hypothesis? The relative importance of these factors may vary based on the invasive species, time-since-invasion, type of impact evaluated, and the particular trait or set of traits chosen to determine invasive species' novelty. Empirical studies are needed to ascertain the relative importance of invader abundance and novelty in these contexts.

This dissertation has exposed the breadth and depth of our current understanding of factors that mediate invasion impacts on soil N pools and fluxes. Confident predictions cannot yet be made regarding which species' additions will be likely to change nitrogen cycling and which ecosystems are most vulnerable, but this work is a significant contribution toward that goal. Using plant functional traits and biogeochemical ecosystem attributes to build a mechanistic understanding of species' effects on ecosystem functioning continues to hold much promise.

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Biography

Marissa Lee was born in 1987 in Concord, Massachusetts and grew up in Apex, North Carolina. When she went off to college in 2005, she got hooked on ecology by participating in research on campus, over summer breaks, and studying abroad in Costa Rica. Marissa received a major in Biology and minor in Religion when she graduated from Swarthmore College in 2009. She also received high honors for her senior thesis on the importance of mycorrhizal fungi for an invasive grass, the same grass species she continued to study for her doctoral dissertation work.

She spent a brief amount of time working as a research technician at Indiana University before returning home to North Carolina to begin her Ph.D. at Duke University in 2010. To support her doctoral research, Marissa was funded through the W.D. Billings Fellowship, the Garden Club of America Wetlands Scholarship, Duke Biology Department Grant-in-Aid of Research Awards, an EPA-Science to Achieve Results Graduate Fellowship, and a NSF-Doctoral Dissertation Improvement Grant. She has published articles in journals such as Biological Invasions and Oecologia and has given numerous talks at professional meetings. She plans to continue investigating the consequences of changing biodiversity in the next stages of her life.