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Invasive species' leaf traits and dissimilarity from natives shape their impact on nitrogen cycling: a meta-analysis

Marissa R. Lee1*, Emily S. Bernhardt1, Peter M. van Bodegom2, J. Hans C. Cornelissen3, Jens Kattge4,5, Daniel C. Laughlin6, Ülo Niinemets7, Josep Peñuelas8,9, Peter B. Reich10,11, Benjamin Yguer12,13, and Justin P. Wright1

1 Department of Biology, Duke University, 130 Science Drive, Durham, NC 27708, USA
2 Institute of Environmental Sciences, Leiden University, Einsteinweg 2, 2333 CC Leiden, the Netherlands
3 Department of Ecological Science, Vrije Universiteit Amsterdam, De Boelelaan 1085, The Netherlands
4 Max Planck Institute for Biogeochemistry, Hans Knöll Str. 10, 07745 Jena, Germany
5 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany
6 Environmental Research Institute, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand
7 Department of Plant Physiology, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
8 CSIC, Global Ecology Unit, CREAF-CSIC-UAB, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain
9 CREAF, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain
10 Department of Forest Resources, University of Minnesota, St Paul, MN 55108, USA
11 Hawkesbury Institute for the Environment, Western Sydney University, Penrith NSW 2751, Australia
12 Unité MECADEV mécanismes adaptatifs et évolution, UMR 7179 CNRS/MNHN, 4 avenue du Petit Château, 91800 Brunoy, France
13 Centre d'Ecologie et des Sciences de la Conservation (CESCO-UMR 7204), Sorbonne Universités-MNHN-CNRS-UPMC, CP51, 55-61 rue Buffon, 75005, Paris, France

* Corresponding author. Email: mrl17@duke.edu; Tel: 919-357-3385
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Summary

- Many exotic species have little apparent impact on ecosystem processes while others have dramatic consequences for human and ecosystem health. There is growing evidence that invasions foster eutrophication. We need to identify species that are harmful and systems that are vulnerable to anticipate these consequences. Species’ traits may provide the necessary insights.

- We conducted a global meta-analysis to determine whether plant leaf and litter functional traits, and particularly leaf and litter nitrogen (N) content and carbon: nitrogen (C : N) ratio, explain variation in invasive species’ impacts on soil N cycling.

- Dissimilarity in leaf and litter traits among invaded and non-invaded plant communities control the magnitude and direction of invasion impacts on N cycling. Invasions that caused the greatest increases in soil inorganic N and mineralization rates had a much greater litter N content and lower litter C : N in the invaded than the reference community. Trait dissimilarities were better predictors than the trait values of invasive species alone.

- Quantifying baseline community tissue traits in addition to those of the invasive species’ is critical to understanding the impacts of invasion on soil N cycling.

Keywords: plant invasions · nitrogen cycling · leaf nitrogen content · leaf carbon to nitrogen · nutrient-use strategy · mineralization · functional trait
Introduction

Globalization has increased rates of invasion by exotic species (Sax et al., 2005) and some have dramatic consequences for human and ecosystem health (Ehrenfeld, 2003; Steffen et al., 2015). A critical way through which species invasions alter ecosystems is by increasing soil nitrogen (N) pools and fluxes (Ehrenfeld, 2003; Liao et al., 2008; Vilà et al., 2011; Pyšek et al., 2012), speeding-up N cycling in ways that can be harmful for people and ecosystems by reducing ecosystem nutrient retention, water quality, carbon storage, and increasing greenhouse gas emissions (Ehrenfeld, 2003; Hickman & Lerdau, 2013). While these biogeochemical changes can be large (i.e. 50% faster N mineralization; Liao et al., 2008), there is significant variability in the direction and magnitude of invasion impacts and we need a better understanding of factors that mediate the adverse impacts of invasion in order to anticipate and remediate the consequences (Pyšek et al., 2012; Hulme et al., 2013).

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 (e.g. N-fixing species), the quality, and the quantity of plant N uptake and release (Knops et al., 2002; Hawkes et al., 2005; Chapman et al., 2006). The role of different plant N uptake and release strategies, as reflected by nitrogen-cycling related leaf and litter traits have not yet been addressed, although previous syntheses have begun to investigate the role of categorical plant traits 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). 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”). N-fixing invasive species have larger soil N effect sizes than non-N-fixing invasive species on average (Liao et al., 2008; Vilà et al., 2011; 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). 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; Phillips et al., 2013; Hobbie, 2015). Thus, non-N fixing species’ impacts on soil N pools and fluxes are likely driven by species differences in nutrient uptake and release pathways (Wedin & Tilman, 1990; Chapman et al., 2006) and moreover, this may be an underappreciated
Plant traits, such as leaf N content and carbon-to-nitrogen (C : N) ratio, are commonly used to characterize how species process nutrients and thus impact soil N cycling (Suding et al., 2008; Makkonen et al., 2012; Reich, 2014). 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 et al., 2010; Ordonez et al., 2010) but see (Funk, 2013). Do these traits drive invasion impacts on N cycling? Evidence from case studies suggests so, especially for non-N-fixing invasive species (Godoy et al., 2009; González-Muñoz et al., 2013). 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 quantified at a global scale.

Traits of the plant community that gets invaded, or the reference community, can have an important influence on the speed, magnitude, and/or direction of N cycling changes following invasion. A previous synthesis supports this hypothesis, finding that invasive N-fixing plants cause stronger impacts on N pools in communities lacking N-fixers (Castro-Díez et al., 2014). The nutrient content and ratio of the reference community, represented by community-weighted mean (CWM) trait values, are likely to be important since they provide information about N cycling pool sizes and flux rates in reference area soils (Reich et al., 2001; Cornwell et al., 2008; Craine et al., 2009; Laughlin, 2011; Freschet et al., 2012) and reflect the cumulative effect of plant species on soil N processes (Grime, 1998; Fortunel et al., 2009), weighted by species’ relative abundance. These baseline levels of soil N cycling 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. However, the roles of reference leaf and litter traits in mediating invasion impacts on soil N cycling are currently unknown.

Trait dissimilarities between an invaded and reference community have the potential to explain the impact of invasion on N cycling as a community shifts to being invader-dominated. Invasive species can impact N cycling via their nutrient-use traits and by 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 (Strickland et al., 2009; Pearse et al., 2013). For example, there is evidence that litter mixtures with a diversity of tissue qualities (e.g. high and low litter N content) undergo decomposition faster than homogenous mixtures due to a complementary suite of resources for decomposing organisms (Gartner & Cardon, 2004). However, other studies found 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; Freschet et al., 2012).

Our objectives in this study are to assess whether the magnitude and direction of N cycling responses to invasion are a function of (1) invasive species’ leaf and litter traits, (2) reference community traits, and (3) trait dissimilarities between invaded and reference communities. To do this, we updated 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 constitute 47% of the full dataset. With this more robust dataset, we test the following hypotheses. We expect that N-fixing invasive species and species with N-rich trait values (e.g. high N content and low C : N) will have greater impacts (Godoy et al., 2009; González-Muñoz et al., 2013). We expect that reference communities with N-fixing species and N-rich CWM trait values will experience more modest N cycling shifts (Reich et al., 2001; Cornwell et al., 2008; Craine et al., 2009; Laughlin, 2011; Castro-Díez et al., 2014). We expect that a large degree of trait dissimilarity between invaded and reference communities will explain large impacts (Gartner & Cardon, 2004; Freschet et al., 2012) and will best predict the direction and magnitude of invasion impacts. 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.

Materials and Methods
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 might 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 (Supporting Information Fig. S1, Notes S1). A total of 67 articles (46.9%) were unique to this meta-analysis dataset (Supporting Information Table S1). 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 the following measurement types: inorganic N pools, ammonification, nitrification, mineralization, or total soil N, and (4) non-invaded reference areas must represent vegetation and soil conditions prior to or in the absence of invasion.

Nitrogen cycling measurements

As in previous meta-analyses (van Kleunen et al., 2010; Vilà et al., 2011; Castro-Díez et al., 2014; Leffler et al., 2014), we extracted all possible invaded and reference area comparisons reported within each article. 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). We identified a total of 404 studies. 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. Measures of inorganic N represent the balance between supply through mineralization and demand from plant roots and soil microbes.

Leaf and litter traits

For all invasive species, the following trait data were collected or estimated: leaf N content, litter N content, leaf C : N, and litter C : N. In cases where there was more than one invasive species in the invaded area, we calculated a weighted average based on species’ relative abundances.

We were also interested in trait CWMs in invaded and reference communities since invaded areas amount to more than simply the traits of the focal invasive species and, similarly, reference areas often include non-native species. Only a small percentage of studies reported trait CWMs (Supporting Information Table S2). If a CWM trait was not reported, we calculated it by combining study-specific species’ lists and relative abundances for the appropriate community (invaded or reference) with trait data from the TRY database (Atkin et al., 1997; Niinemets, 1999; Shipley & Lechowicz, 2000; White et al., 2000; Niinemets, 2001; Ogaya & Penuelas, 2003; Quested et al., 2003; Wright et al., 2004; Craine et al., 2005; Louault et al., 2005; Han et al., 2005; Preston et al., 2006; Bakker et al., 2006; Kerkhoff et al., 2006; Kattge et al., 2009; Freschet et al., 2010; Laughlin et al., 2010; Willis et al., 2010; Kattge et al., 2011; Wright et al., 2011). Some studies provided species’ lists but did not measure species’ relative abundances in invaded and reference areas. In these cases, we extracted relative abundances from authors’ in-text descriptions (e.g. “invader cover was approximately 80%”) or, lacking any clues, assumed that species were present in equal relative abundance. The assumption was necessary for 19% (leaf N content), 22% (leaf C : N), 45% (litter N content), 21% (litter C : N) of the CWM trait values on average (Supporting Information Table S3).

Since we needed to estimate many trait CWM values, we assigned each value a quality rank to summarize number of assumptions that entered into the estimate. CWM trait value estimates require knowledge of (1) species’ relative abundances in the community and (2) species’ mean trait values. Trait data were assigned to species using the highest quality data available from
among the following sources (high to low quality): species’ mean study-reported trait estimate, species’ mean TRY database trait estimate, and genus’ mean TRY database trait estimate (Supporting Information Table S3). Genus-level data can be a useful approximation of species’ trait values in their absence (Shan et al., 2012; Schrodt et al., 2015). As such, these estimates are lower in quality if a large proportion of species present in the community (1a) do not have species-specific associated relative abundance data, (1b) do not have any associated relative abundance data, or (2) do not have species-specific trait data. To qualitatively evaluate the significance of CWM data quality in our analyses, we computed a quality rank for each CWM trait value. Values that were reported in the original study are considered highest quality and were assigned a quality rank of four. Values that were estimated were assigned one point if more than 25% of species within the community do not have the low quality characteristics listed above (1a, 1b, or 2; Supporting Information Table S3). 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. We were able to obtain leaf N content trait values and CWMs for the majority of invasive species, and invaded and reference communities (Supporting Information Table S3, Fig. S2), whereas it was more difficult to assign trait values and CWMs for the other traits: leaf C : N, litter N content, and litter C : N (Supporting Information Table S3, Fig. S2).

Trait dissimilarities

Trait dissimilarities were calculated as the univariate difference in CWMs, invaded minus reference, such that higher trait dissimilarity values indicate higher trait values in the invaded than the reference community. Like CWM trait values, CWM trait dissimilarity values were each assigned a quality rank to summarize number of assumptions that entered into the estimate.

For each CWM trait dissimilarity value, the quality rank was calculated as simply the sum of the CWM trait value quality ranks for the invaded and reference communities (refer to the Leaf and litter traits section) that were used to calculate the trait dissimilarity value, resulting in a maximum quality rank of eight. To determine the importance of calculating trait dissimilarity using the invaded CWM or the invasive species trait value for post-hoc analyses, we calculated
the dissimilarity between the invasive species’ trait value and the reference CWM. This results in equivalent dissimilarity values for studies where the invaded community was composed of only invasive species (56% of this dataset),

Other factors

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. N-fixing status included four levels: no N-fixers, reference community N-fixers only, invasive species N-fixers only, and reference and invasive species N-fixers (Supporting Information Table S2). Study type included four levels: field observation, field addition, field removal, and greenhouse study (Supporting Information Table S2). 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/).

Regarding study design, a previous meta-analysis found that whether the study was observational, experimental, or a combination does not change our ability to detect the impacts of invasion on N cycling. It is important to continue to monitor study design as a potential signal as we add new studies since (i) observational studies are subject to the influence of environmental filtering and are often measuring the effects of long term invasion, (ii) experimental results are contingent on whether invaders are added (Lee et al., 2012) or removed (DeMeester & deB Richter, 2010), and (ii) greenhouse studies are constrained by pot sizes, duration, and lack of community effects such as litter accumulation (Lee et al., 2012).

Statistical analyses

Invasion effect sizes were calculated using Hedges’ d effect-size statistics such that reference values were subtracted from invaded area values (Hedges, 1981; Viechtbauer, 2010) (Supporting Information Methods S1). This effect size metric 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. Calculations were done using the escalc function with measure=“SMD” in the metafor package of R (Viechtbauer, 2010). Effect sizes greater than zero indicate that measurement values are higher in invaded than reference areas.
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 (Supporting Information Methods S2). Each model included nested random effects of study within paper to account for the structure of the dataset (Supporting Information Methods S2). 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 $\chi^2$ 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 the grand effect sizes, we created models to test the significance of the following fixed effects: (a) invasive species’ leaf or litter traits, (b) reference CWMs, (c) dissimilarities between invaded and reference CWMs, (d) N-fixing status, and (e) study design (Supporting Information Methods S2). We did not construct models with multiple fixed effects because of the abundance of missing data across studies. We identified models with a significant fixed effect based on a test of residual heterogeneity and accounted for multiple comparisons based on trait type using alpha equal to 0.1 and a sequential Holm-Bonferroni procedure (Holm, 1979). 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 (Supporting Information Methods S3). For models with a significant fixed effect, we evaluated whether the quality of trait data in that analysis may have biased our findings by regressing trait quality ranks against invasion effect sizes and visually inspecting data by quality rank in the trait-by-effect size scatterplots. For models that included trait dissimilarity as a significant fixed effect, we replaced the trait dissimilarity value with one that was calculated using invasive species’ trait values instead of the invaded CWMs and re-ran the model to evaluate whether our findings depend upon non-invasive species that are present in the invaded community. 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 et al., 2013). For each effect type, we evaluated publication bias in our dataset by testing for correlation among study effect sizes and sample sizes, looking for asymmetry in funnel plots (Supporting Information Fig. S3), 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 any 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 (Supporting Information Table S4).

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).

Results

Global effect sizes

On average, invaded areas have significantly larger ammonium pools (+49%), faster nitrification (+371%), faster mineralization rates (+105%), more total soil N (+65%), and more soil organic matter (+37%) than reference areas (Table 1, all p<.05). Total soil inorganic N tends to be larger in invaded areas (+94%) but is not significantly different from zero (Table 1). Ammonification rates and soil C : N values do not significantly differ in invaded relative to reference areas (Table 1).

Invasive species traits

Of the four types of invasive species traits (leaf N content, litter N content, leaf C : N, and litter C : N) and nine soil N effect sizes, one trait-by-effect size correlation is significant (Table 2; Supporting Information Fig. S4). Average invasive species’ traits 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 (Supporting Information Fig. S2). Invasion increases soil N to greater extents when the invasive species has high leaf N content. CWM value quality was not a significant factor in mediating invasion effect sizes for any of the meta-regression models with a significant fixed effect and visual inspection of scatterplots did not
reveal a quality bias (Supporting Information Fig. S4). Moreover, this was the case for all significant models that included CWM trait data (Fig. 1).

Reference CWMs

Of the four types of reference CWM traits and nine soil N effect sizes, none of the trait-by-effect size correlations are significant (Table 2). Average 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 (Supporting Information Fig. S2).

Trait dissimilarities

Of the four trait dissimilarities among invaded and reference area communities (Inv. – Ref.) and nine soil N effect sizes, four trait-by-effect size correlations are significant (Table 2; Fig. 1). Average trait dissimilarities are +0.38±0.04% leaf N and +0.19±0.07% litter N, +0.84±1.15 leaf C : N, and -17.47±4.13 litter C : N (Supporting Information Fig. S2). Invasion increases total inorganic N pools to greater extents when the invaded community has much greater litter N content and lower litter C : N than the reference community (Fig. 1). Invasion increases mineralization rates and decreases soil C : N values to greater extents when the invaded community has much lower leaf C : N than the reference community (Fig. 1). Similar results were obtained when trait dissimilarity values were re-calculated with invasive species’ trait values instead of invaded CWM trait values (Supporting Information Table S5).

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, Supporting Information Table S2). 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 (Supporting Information Table S2). A study’s N-fixing status is important for explaining three soil nitrogen invasion effect sizes: total inorganic N, soil N, and soil C : N (Fig. 2). 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, 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 (Fig. 2). 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.

Study design
Observational field studies are by far the most frequent type of study in this dataset (273 of 404 studies, Supporting Information Table S2). Study design is a significant predictor for nitrate and soil organic matter effect sizes (Fig. 3). 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 (Fig. 3). 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 (Fig. 3). Only one experimental addition study measured soil organic matter.

Discussion
Invasions influence soil N cycling, fostering ecosystem eutrophication across the globe; however, the extents to which this happens in the presence of invasive species and reference plant communities with N-rich or N-poor leaf and litter tissue has been unknown until now. Using a traits-based approach, our meta-analysis provides insight into importance of leaf and litter traits as drivers of invasive species’ impacts on soil N cycling. Our synthesis shows that the likelihood of an invader enhancing N cycling depends strongly upon how different that invaders’ pattern of nutrient uptake and release is from the community it invades. Invasive species with higher leaf N content increase soil N in invaded areas to greater extents. Moreover, dissimilarity in CWM traits among invaded and reference communities performs better than raw CWM trait values at explaining variability in soil N cycling. Invasions that fostered the greatest increases in total inorganic N and mineralization rates are invasions in which the invaded community has much greater litter N content and lower leaf and litter C : N than the reference community. Given these results, future studies should devote more attention to gathering community trait metrics
and to quantifying reference or “baseline” community traits in areas that are vulnerable to invasion.

Global effect sizes
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 where the invasive species is a non-N-fixing species and the reference community contains an N-fixing species (16 studies). 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.

Invasive species’ leaf and litter traits
When apparent, the associations we detected 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 content and low C : N), whereas more modest shifts in N cycling occurred where the reference community had N-rich CWM trait values. Only one invasive species’ plant trait (leaf N content) explained variation in the magnitude and direction of changes in soil N due to invasion. In this case where an invader trait could explain invasion effect sizes on a soil property, 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 content, explained variation in bulk soil properties like soil N and soil C
It is impossible to tell 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. Variability in the extent to which these traits are informative could have biological significance since particular traits 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 content may be a more important driver of its impact on soil inorganic N than that of Morella (Myrica) 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 (e.g. mycorrhizal association type or root exudation rate) may have more explanatory power for some invasive species and contexts (Bardgett et al., 2014), but are more rarely measured or reported. And finally, another reason that some traits and effect sizes may not be strongly correlated is because soil N processes are also regulated by abiotic factors such as soil type, moisture, and temperature which can obscure expected relationships between plant nutrient-use traits and soil N values (Castro-Díez et al., 2014; Hobbie, 2015).

Reference community leaf and litter traits
There is limited evidence that reference community characteristics besides N-fixing status 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 CWMs alone were very strong predictors of invader effects on N cycling. Reference species traits did not contain sufficient information to explain invasion effects on soil N pools or fluxes. 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.

Trait dissimilarities
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 CWM trait estimates from the
invaded and reference areas so as to reflect the way that invasion impacts on ecosystem
processes are calculated (Garnier et al., 2004; Díaz et al., 2007). Some field studies use CWM
traits to explain variation in soil N cycling in invaded and reference areas, 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. Whereas leaf N content was the most informative
invasive species’ traits, the dissimilarity in C : N ratio between invaded and reference
communities better explained the impact of invasion on soil N pools and fluxes. 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 content alone.

Study design

On the whole, the influence of study design on the magnitude and direction of invasion effect
sizes was limited with only 2 of 9 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 et al. (2014)’s finding that removal studies have smaller N pool and flux effect sizes than studies with a non-invaded reference, 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 point to the need to 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 will 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.
Acknowledgements

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Author Contributions

M.L., E.B., and J.W. developed the study idea and analyses. M.L. built the meta-analysis dataset and conducted the analyses using data produced by P.B., J.H.C., J.K., D.L., U.N., J.P., P.R., and B.Y. M.L. wrote the manuscript with assistance from all co-authors.
References


**Smith VC, Bradford MA. 2003.** Do non-additive effects on decomposition in litter-mix experiments result from differences in resource quality between litters? *Oikos 102:* 235–242.


Tables

Table 1. 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.

<table>
<thead>
<tr>
<th>Soil measurement</th>
<th>This study</th>
<th>Liao et al. 2008</th>
<th>Vila et al. 2011</th>
<th>Castro-Diez et al. 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect size (d*)</td>
<td>95% CI</td>
<td>k</td>
<td>Effect size (RR++)</td>
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<tr>
<td>Inorganic N pool</td>
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<td>0.12 to 0.40</td>
<td>141</td>
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<td>NO₃⁻</td>
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<td>-0.003 to 0.34</td>
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<td></td>
<td>NH₄⁺+NO₃⁻</td>
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<td>-0.05 to 0.35</td>
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<td>N flux</td>
<td>Δ NH₄⁺</td>
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<td>-0.31 to 0.20</td>
<td>50</td>
</tr>
<tr>
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<td>Δ NO₃⁻</td>
<td>0.45</td>
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<tr>
<td></td>
<td>Δ NH₄⁺+NO₃⁻</td>
<td>0.36</td>
<td>0.14 to 0.58</td>
<td>101</td>
</tr>
<tr>
<td>Other</td>
<td>Soil N</td>
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<td>0.31 to 0.75</td>
<td>187</td>
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<tr>
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<td>Soil C : N</td>
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<td>-0.25 to 0.14</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SOM</td>
<td>0.59</td>
<td>0.18 to 0.99</td>
<td>90</td>
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</table>
Table 2. Summary of mixed effects models used to explain invasion effect sizes (response variable) using invasive species’ trait values, reference community-weighted mean (CWM) trait values, or dissimilarity between invaded and reference CWMs 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 a significant fixed effect after Holm-Bonferroni corrections for multiple comparisons are highlighted in bold grey.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Trait type</th>
<th>Hyp.</th>
<th>Invasive species</th>
<th>Reference CWM</th>
<th>CWM Dissimilarity</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Leaf</td>
<td>Litter</td>
<td>Leaf</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>est</td>
<td>$R_p^2$</td>
<td>k</td>
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<tr>
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<td>NH₄⁺</td>
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<td>0.01</td>
<td>0.00</td>
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</tr>
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<td></td>
<td></td>
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<td>k</td>
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<tr>
<td></td>
<td>NO₃⁻</td>
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<td>NH₄⁺+NO₃⁻</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>est</td>
<td>$R_p^2$</td>
<td>k</td>
</tr>
<tr>
<td></td>
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<td>0.00</td>
<td>67</td>
</tr>
<tr>
<td></td>
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<td>0.00</td>
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<tr>
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<td>0.09</td>
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<td>0.71</td>
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<td>Other</td>
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<td>64</td>
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<tr>
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<td>75</td>
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<tr>
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<td>SOM</td>
<td>+</td>
<td>0.01</td>
<td>0.00</td>
<td>97</td>
</tr>
<tr>
<td>Inorganic N pool</td>
<td>NH₄⁺</td>
<td>+</td>
<td>-0.01</td>
<td>0.00</td>
<td>19</td>
</tr>
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<td>-</td>
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<td>0.00</td>
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<tr>
<td>N flux</td>
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<td></td>
<td>NO₃⁻</td>
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<td>NA</td>
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<td>NH₄⁺+NO₃⁻</td>
<td>+</td>
<td>0.03</td>
<td>0.00</td>
<td>28</td>
</tr>
</tbody>
</table>

NA indicates that the model did not converge
**Figure Legends**

**Figure 1.** Community-weighted mean (CWM) trait dissimilarities between invaded and reference plant communities shape the magnitude and direction of invasion impacts on aspects of soil N cycling. **(a)** Greater litter N content and **(b)** lower litter C : N in invaded than reference communities is associated with larger increases in total inorganic N due to invasion. Greater leaf C : N in invaded than reference communities is associated with **(c)** smaller increases and even decreases in mineralization and **(d)** smaller declines and even increases in soil C : N. Point size is scaled by study variance such that larger points have smaller variance. Point shade represents CWM quality rank such that darker points are higher in quality. Trait dissimilarity is a significant fixed effect in all regression models shown. Solid line and blue shaded region show the meta-regression model fit and the 95% confidence interval; see Table 2 for fit statistics. 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 2.** Presence of N-fixing plant species shapes the magnitude and direction of invasion impacts on total inorganic N, soil N, and soil C : N effect sizes. 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; mean +/- 95% confidence interval with the number of studies shown in parentheses.

**Figure 3.** Study design mediates soil nitrate and organic matter invasion effect sizes. 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; mean +/- 95% confidence interval with the number of studies shown in parentheses.
Supporting Information Legends

Figure S1 Flow chart of the article identification and selection process.

Figure S2 Frequency distribution of invasive species’ trait values and CWM trait dissimilarities.

Figure S3 Effect size funnel plots.

Figure S4 Invasive species’ with higher leaf %N promote greater increases in soil N.

Table S1 List of the 143 articles used in our meta-analysis on the impacts of invasion on soil N cycling and the type of data extracted.

Table S2. Predictor variables used to explain variation in invasion impacts on soil N cycling in this study.

Table S3 Summary of trait data quality by community and trait type.

Table S4 Impact of invasion on soil N cycling with associated publication bias checks and Q statistics.

Table S5 Mixed effects model results that included trait dissimilarity as a significant fixed effect (Invaded – Reference CWM) and the results of re-running that model with trait dissimilarity calculated as the invasive species trait value minus the reference CWM (Invasive sp. – Reference CWM).

Methods S1 Hedges’ d calculation.

Methods S2 R code for meta-analysis and meta-regression models.

Methods S3 Pseudo-R² calculation.
Notes S1 Invasion impacts on soil nitrogen cycling references.