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

3

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32

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34

35

36 **Summary**

- 37 • Many exotic species have little apparent impact on ecosystem processes while others
38 have dramatic consequences for human and ecosystem health. There is growing evidence
39 that invasions foster eutrophication. We need to identify species that are harmful and
40 systems that are vulnerable to anticipate these consequences. Species' traits may provide
41 the necessary insights.
- 42 • We conducted a global meta-analysis to determine whether plant leaf and litter functional
43 traits, and particularly leaf and litter nitrogen (N) content and carbon: nitrogen (C : N)
44 ratio, explain variation in invasive species' impacts on soil N cycling.
- 45 • Dissimilarity in leaf and litter traits among invaded and non-invaded plant communities
46 control the magnitude and direction of invasion impacts on N cycling. Invasions that
47 caused the greatest increases in soil inorganic N and mineralization rates had a much
48 greater litter N content and lower litter C : N in the invaded than the reference
49 community. Trait dissimilarities were better predictors than the trait values of invasive
50 species alone.
- 51 • Quantifying baseline community tissue traits in addition to those of the invasive species'
52 is critical to understanding the impacts of invasion on soil N cycling.

53

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

56

57 **Introduction**

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

69

70 Invasive species can impact soil N cycling through a variety of mechanisms. For example, they
71 can alter the activity or composition of microbial N-transformers (e.g. N-fixing species), the
72 quality, and the quantity of plant N uptake and release (Knops *et al.*, 2002; Hawkes *et al.*, 2005;
73 Chapman *et al.*, 2006). The role of different plant N uptake and release strategies, as reflected by
74 nitrogen-cycling related leaf and litter traits have not yet been addressed, although previous
75 syntheses have begun to investigate the role of categorical plant traits and climate conditions in
76 mediating the impacts of invasion on soil processes (Liao *et al.*, 2008; Vilà *et al.*, 2011; Castro-
77 Díez *et al.*, 2014). The mediating factor that has received most attention, with good reason, has
78 been whether the invasive species associates with N-fixing bacteria (hereafter, “N-fixing
79 species”). N-fixing invasive species have larger soil N effect sizes than non-N-fixing invasive
80 species on average (Liao *et al.*, 2008; Vilà *et al.*, 2011; Castro-Díez *et al.*, 2014). However, even
81 non-N-fixing invasive species are associated with increases in soil N pools and fluxes (Liao *et*
82 *al.*, 2008; Castro-Díez *et al.*, 2014). Plant species can mediate soil N pools and fluxes by having
83 either fast nutrient uptake and release, promoting fast N cycling, or slow nutrient uptake and
84 release, promoting slow N cycling (Knops *et al.*, 2002; Chapman *et al.*, 2006; Laungani &
85 Knops, 2009; Phillips *et al.*, 2013; Hobbie, 2015). Thus, non-N fixing species’ impacts on soil N
86 pools and fluxes are likely driven by species differences in nutrient uptake and release pathways
87 (Wedin & Tilman, 1990; Chapman *et al.*, 2006) and moreover, this may be an underappreciated

88 component of N-fixing species' impacts.

89

90 Plant traits, such as leaf N content and carbon-to-nitrogen (C : N) ratio, are commonly used to
91 characterize how species process nutrients and thus impact soil N cycling (Suding *et al.*, 2008;
92 Makkonen *et al.*, 2012; Reich, 2014). There is evidence across the globe that invasive species
93 have leaf traits that indicate that they have faster nutrient-use strategies than native species (van
94 Kleunen *et al.*, 2010; Ordonez *et al.*, 2010) but see (Funk, 2013). Do these traits drive invasion
95 impacts on N cycling? Evidence from case studies suggests so, especially for non-N-fixing
96 invasive species (Godoy *et al.*, 2009; González-Muñoz *et al.*, 2013). However, the role of
97 invasive species' leaf and litter traits in driving the direction and magnitude of invasion impacts
98 on soil N cycling has not been quantified at a global scale.

99

100 Traits of the plant community that gets invaded, or the reference community, can have an
101 important influence on the speed, magnitude, and/or direction of N cycling changes following
102 invasion. A previous synthesis supports this hypothesis, finding that invasive N-fixing plants
103 cause stronger impacts on N pools in communities lacking N-fixers (Castro-Díez *et al.*, 2014).
104 The nutrient content and ratio of the reference community, represented by community-weighted
105 mean (CWM) trait values, are likely to be important since they provide information about N
106 cycling pool sizes and flux rates in reference area soils (Reich *et al.*, 2001; Cornwell *et al.*, 2008;
107 Craine *et al.*, 2009; Laughlin, 2011; Freschet *et al.*, 2012) and reflect the cumulative effect of
108 plant species on soil N processes (Grime, 1998; Fortunel *et al.*, 2009), weighted by species'
109 relative abundance. These baseline levels of soil N cycling may be extreme enough that increases
110 or decreases are impossible because of biogeochemical constraints. For example, the increases in
111 N cycling are less likely for systems with extremely N-rich reference communities since invasive
112 species' traits cannot be more N-rich. However, the roles of reference leaf and litter traits in
113 mediating invasion impacts on soil N cycling are currently unknown.

114

115 Trait dissimilarities between an invaded and reference community have the potential to explain
116 the impact of invasion on N cycling as a community shifts to being invader-dominated. Invasive
117 species can impact N cycling via their nutrient-use traits and by displacing native plant species
118 that are likewise important in shaping N pools and fluxes. Trait dissimilarity addresses both of

119 those impact pathways. In addition, a third mechanism of trait dissimilarity effects is that new
120 combinations of trait values may have synergistic impacts on N cycling. Whether the addition of
121 litter that is much higher in quality promotes larger than expected or smaller than expected
122 impacts on net N mineralization due to microbial community mediation is the subject of
123 continued debate (Strickland *et al.*, 2009; Pearse *et al.*, 2013). For example, there is evidence that
124 litter mixtures with a diversity of tissue qualities (e.g. high and low litter N content) undergo
125 decomposition faster than homogenous mixtures due to a complementary suite of resources for
126 decomposing organisms (Gartner & Cardon, 2004). However, other studies found that
127 decomposition is more rapid for litter mixtures that most closely match the litter quality to which
128 the decomposing organisms are accustomed (e.g. high or low litter N), exhibiting a ‘home-field
129 advantage’ (Smith & Bradford, 2003; Strickland *et al.*, 2009; Freschet *et al.*, 2012).

130

131 Our objectives in this study are to assess whether the magnitude and direction of N cycling
132 responses to invasion are a function of (1) invasive species’ leaf and litter traits, (2) reference
133 community traits, and (3) trait dissimilarities between invaded and reference communities. To do
134 this, we updated the previous meta-analyses on N cycling responses to invasion (Liao *et al.*,
135 2008; Vilà *et al.*, 2011; Castro-Díez *et al.*, 2014) with 67 newly added publications, which
136 constitute 47% of the full dataset. With this more robust dataset, we test the following
137 hypotheses. We expect that N-fixing invasive species and species with N-rich trait values (e.g.
138 high N content and low C : N) will have greater impacts (Godoy *et al.*, 2009; González-Muñoz *et al.*,
139 2013). We expect that reference communities with N-fixing species and N-rich CWM trait
140 values will experience more modest N cycling shifts (Reich *et al.*, 2001; Cornwell *et al.*, 2008;
141 Craine *et al.*, 2009; Laughlin, 2011; Castro-Díez *et al.*, 2014). We expect that a large degree of
142 trait dissimilarity between invaded and reference communities will explain large impacts
143 (Gartner & Cardon, 2004; Freschet *et al.*, 2012) and will best predict the direction and magnitude
144 of invasion impacts. Addressing these aims will clarify how, on a global scale, plant invasions
145 impact soil N cycling and which factors mediate the direction and magnitude of those impacts.

146

147

148 **Materials and Methods**

149

150 *Study selection*

151 The aim of the literature search was to collect observational and experimental studies that
152 measure soil nitrogen properties in invaded and reference areas. We evaluated papers cited in
153 Liao *et al.* 2008 and conducted database searches to collect articles published after 2007 and any
154 articles published prior to 2007 that might have been overlooked. The search terms for the two
155 Web of Science queries that were conducted on 11/17/14 were (1) (("invasion" OR "invasive")
156 AND ("nitrogen cycling" OR "soil nitrogen") AND ("plant" OR "grass")) and (2) (("invasion"
157 OR "invasive") AND ("soil nitrogen" OR "nitrogen cycling" OR "soil nutrient")). Related
158 records were also identified if they were cited within any of the full-text articles assessed for
159 eligibility, but were not captured in the literature search. Of the 483 unique articles that were
160 identified, a total of 143 articles were acceptable for inclusion in this meta-analysis (Supporting
161 Information Fig. S1, Notes S1). A total of 67 articles (46.9%) were unique to this meta-analysis
162 dataset (Supporting Information Table S1). The following criteria were used to identify
163 acceptable articles: (1) article must present data on the relationship between an invasive plant
164 species or set of invasive species (up to 5 species) and soil nitrogen measurements, (2)
165 measurements must be taken in both an invaded and a reference area, (3) must include at least
166 one of the following measurement types: inorganic N pools, ammonification, nitrification,
167 mineralization, or total soil N, and (4) non-invaded reference areas must represent vegetation and
168 soil conditions prior to or in the absence of invasion.

169

170 *Nitrogen cycling measurements*

171 As in previous meta-analyses (van Kleunen *et al.*, 2010; Vilà *et al.*, 2011; Castro-Díez *et al.*,
172 2014; Leffler *et al.*, 2014), we extracted all possible invaded and reference area comparisons
173 reported within each article. Each unique comparison we termed a “study”. Most articles
174 included more than one study, so we accounted for this nested quality of the data structure in our
175 meta-analysis models. Studies within the same article were considered distinct if they presented
176 data from different study types (e.g. field observation and greenhouse study) or sites. Data from
177 repeated measures were aggregated as in Liao *et al.* (2008). We identified a total of 404 studies.

178

179 As available, the following soil measurement data from within invaded and reference plots were
180 collected from each study: soil inorganic N pool concentrations (ammonium, nitrate, total

181 inorganic N), net or gross inorganic N mineralization rates (ammonification, nitrification,
182 mineralization), soil organic matter, total soil N, and soil C : N. Measures of inorganic N
183 represent the balance between supply through mineralization and demand from plant roots and
184 soil microbes.

185

186 *Leaf and litter traits*

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

190

191 We were also interested in trait CWMs in invaded and reference communities since invaded
192 areas amount to more than simply the traits of the focal invasive species and, similarly, reference
193 areas often include non-native species. Only a small percentage of studies reported trait CWMs
194 (Supporting Information Table S2). If a CWM trait was not reported, we calculated it by
195 combining study-specific species' lists and relative abundances for the appropriate community
196 (invaded or reference) with trait data from the TRY database (Atkin *et al.*, 1997; Niinemets,
197 1999; Shipley & Lechowicz, 2000; White *et al.*, 2000; Niinemets, 2001; Ogaya & Penuelas,
198 2003; Quested *et al.*, 2003; Wright *et al.*, 2004; Craine *et al.*, 2005; Louault *et al.*, 2005; Han *et*
199 *al.*, 2005; Preston *et al.*, 2006; Bakker *et al.*, 2006; Kerkhoff *et al.*, 2006; Kattge *et al.*, 2009;
200 Freschet *et al.*, 2010; Laughlin *et al.*, 2010; Willis *et al.*, 2010; Kattge *et al.*, 2011; Wright *et al.*,
201 2011). Some studies provided species' lists but did not measure species' relative abundances in
202 invaded and reference areas. In these cases, we extracted relative abundances from authors' in-
203 text descriptions (e.g. "invader cover was approximately 80%") or, lacking any clues, assumed
204 that species were present in equal relative abundance. The assumption was necessary for 19%
205 (leaf N content), 22% (leaf C : N), 45% (litter N content), 21% (litter C : N) of the CWM trait
206 values on average (Supporting Information Table S3).

207

208 Since we needed to estimate many trait CWM values, we assigned each value a quality rank to
209 summarize number of assumptions that entered into the estimate. CWM trait value estimates
210 require knowledge of (1) species' relative abundances in the community and (2) species' mean
211 trait values. Trait data were assigned to species using the highest quality data available from

212 among the following sources (high to low quality): species' mean study-reported trait estimate,
213 species' mean TRY database trait estimate, and genus' mean TRY database trait estimate
214 (Supporting Information Table S3). Genus-level data can be a useful approximation of species'
215 trait values in their absence (Shan *et al.*, 2012; Schrodte *et al.*, 2015). As such, these estimates are
216 lower in quality if a large proportion of species present in the community (1a) do not have
217 species-specific associated relative abundance data, (1b) do not have any associated relative
218 abundance data, or (2) do not have species-specific trait data. To qualitatively evaluate the
219 significance of CWM data quality in our analyses, we computed a quality rank for each CWM
220 trait value. Values that were reported in the original study are considered highest quality and
221 were assigned a quality rank of four. Values that were estimated were assigned one point if more
222 than 25% of species within the community do not have the low quality characteristics listed
223 above (1a, 1b, or 2; Supporting Information Table S3). CWM trait values were only included in
224 our analyses if the observation had at least one invasive and reference species, with each species
225 having a mean trait value for the trait of interest and estimates for the species' abundances in the
226 invaded area and reference area. We were able to obtain leaf N content trait values and CWMs
227 for the majority of invasive species, and invaded and reference communities (Supporting
228 Information Table S3, Fig. S2), whereas it was more difficult to assign trait values and CWMs
229 for the other traits: leaf C : N, litter N content, and litter C : N (Supporting Information Table S3,
230 Fig. S2).

231

232 *Trait dissimilarities*

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

237

238 For each CWM trait dissimilarity value, the quality rank was calculated as simply the sum of the
239 CWM trait value quality ranks for the invaded and reference communities (refer to the *Leaf and*
240 *litter traits* section) that were used to calculate the trait dissimilarity value, resulting in a
241 maximum quality rank of eight. To determine the importance of calculating trait dissimilarity
242 using the invaded CWM or the invasive species trait value for post-hoc analyses, we calculated

243 the dissimilarity between the invasive species' trait value and the reference CWM. This results in
244 equivalent dissimilarity values for studies where the invaded community was composed of only
245 invasive species (56% of this dataset),

246

247 *Other factors*

248 We were also interested in the role of N-fixing species' presence in invaded and reference areas
249 (hereafter "N-fixing status") and study design as factors that mediate the impact of invasive
250 plants on soil N cycling. Therefore, we categorized studies into an N-fixing status and study
251 type. N-fixing status included four levels: no N-fixers, reference community N-fixers only,
252 invasive species N-fixers only, and reference and invasive species N-fixers (Supporting
253 Information Table S2). Study type included four levels: field observation, field addition, field
254 removal, and greenhouse study (Supporting Information Table S2). N-fixing species were
255 identified using the study-specific species lists and a list of *Frankia*-associated and Actinorhizal
256 plant genera from The Plant List (accessed May 11, 2015, <http://www.theplantlist.org/>).

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

265

266 *Statistical analyses*

267 Invasion effect sizes were calculated using Hedges' d effect-size statistics such that reference
268 values were subtracted from invaded area values (Hedges, 1981; Viechtbauer, 2010) (Supporting
269 Information Methods S1). This effect size metric was chosen instead of the log response ratio
270 used by Liao *et al.* (2008) because N mineralization measurements are often measured as net
271 rates and thus can take on negative values. Calculations were done using the *escalc* function with
272 measure="SMD" in the *metafor* package of R (Viechtbauer, 2010). Effect sizes greater than zero
273 indicate that measurement values are higher in invaded than reference areas.

274
275 We estimated a grand effect size for each soil measurement type in our analyses using linear
276 mixed models, performed with the *rma.mv* function and the REML (restricted maximum
277 likelihood) method in the *metafor* package (Supporting Information Methods S2). Each model
278 included nested random effects of study within paper to account for the structure of the dataset
279 (Supporting Information Methods S2). Study effect sizes were evaluated for normality to address
280 model assumptions. In the context of this model, the estimated intercept parameter represents the
281 estimated grand effect size. A test for residual heterogeneity was carried out using the χ^2
282 distribution to determine whether variability in the observed effect sizes or outcomes is larger
283 than one would expect based on sampling variability. Parameter estimates, 95% confidence
284 intervals, and variance components were also obtained from *rma.mv* output.

285
286 To evaluate the role of factors that may mediate the grand effect sizes, we created models to test
287 the significance of the following fixed effects: (a) invasive species' leaf or litter traits, (b)
288 reference CWMs, (c) dissimilarities between invaded and reference CWMs, (d) N-fixing status,
289 and (e) study design (Supporting Information Methods S2). We did not construct models with
290 multiple fixed effects because of the abundance of missing data across studies. We identified
291 models with a significant fixed effect based on a test of residual heterogeneity and accounted for
292 multiple comparisons based on trait type using alpha equal to 0.1 and a sequential Holm-
293 Bonferroni procedure (Holm, 1979). We evaluated the model fit by calculating a pseudo- R^2
294 value based on the proportion of variance explained in the full model relative to a reduced model
295 without the fixed effect (Supporting Information Methods S3). For models with a significant
296 fixed effect, we evaluated whether the quality of trait data in that analysis may have biased our
297 findings by regressing trait quality ranks against invasion effect sizes and visually inspecting
298 data by quality rank in the trait-by-effect size scatterplots. For models that included trait
299 dissimilarity as a significant fixed effect, we replaced the trait dissimilarity value with one that
300 was calculated using invasive species' trait values instead of the invaded CWMs and re-ran the
301 model to evaluate whether our findings depend upon non-invasive species that are present in the
302 invaded community. For categorical fixed effects such as N-fixing status and study design, we
303 ran post-hoc Wald-type tests to evaluate differences among factor levels.

304

305 Meta-analyses can be subject to publication bias (Koricheva *et al.*, 2013). For each effect type,
306 we evaluated publication bias in our dataset by testing for correlation among study effect sizes
307 and sample sizes, looking for asymmetry in funnel plots (Supporting Information Fig. S3), and
308 calculating the fail-safe number (Koricheva *et al.*, 2013; Castro-Díez *et al.*, 2014). We did not
309 detect a significant correlation among study effect sizes and sample sizes for any effect types
310 except total inorganic N effect sizes, which suggests that larger effects sizes in one direction are
311 not more likely to be published for most effect types (Supporting Information Table S4).

312
313 All statistical analyses were conducted using R Statistical Software (R Development Core Team
314 and Team 2012). The R packages plyr and ggplot2 were used to manipulate and visualize data
315 (Wickham 2009, 2011).

318 **Results**

320 *Global effect sizes*

321 On average, invaded areas have significantly larger ammonium pools (+49%), faster nitrification
322 (+371%), faster mineralization rates (+105%), more total soil N (+65%), and more soil organic
323 matter (+37%) than reference areas (Table 1, all $p < .05$). Total soil inorganic N tends to be larger
324 in invaded areas (+94%) but is not significantly different from zero (Table 1). Ammonification
325 rates and soil C : N values do not significantly differ in invaded relative to reference areas (Table
326 1).

328 *Invasive species traits*

329 Of the four types of invasive species traits (leaf N content, litter N content, leaf C : N, and litter
330 C : N) and nine soil N effect sizes, one trait-by-effect size correlation is significant (Table 2;
331 Supporting Information Fig. S4). Average invasive species' traits are $2.3 \pm 0.1\%$ leaf N, $1.2 \pm 0.1\%$
332 litter N, 28.7 ± 1.0 leaf C : N, and 54.2 ± 4.7 litter C : N (Supporting Information Fig. S2). Invasion
333 increases soil N to greater extents when the invasive species has high leaf N content. CWM
334 value quality was not a significant factor in mediating invasion effect sizes for any of the meta-
335 regression models with a significant fixed effect and visual inspection of scatterplots did not

336 reveal a quality bias (Supporting Information Fig. S4). Moreover, this was the case for all
337 significant models that included CWM trait data (Fig. 1).

338

339 *Reference CWMs*

340 Of the four types of reference CWM traits and nine soil N effect sizes, none of the trait-by-effect
341 size correlations are significant (Table 2). Average reference CWMs are $1.7 \pm 0.0\%$ leaf N,
342 $1.0 \pm 0.1\%$ litter N, 31.6 ± 1.2 leaf C : N, and 77.9 ± 5.3 litter C : N (Supporting Information Fig.
343 S2).

344

345 *Trait dissimilarities*

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

356

357 *Presence of N-fixing species*

358 Most observations do not include an N-fixing species in the reference plant community or as the
359 focal invasive species (295 of 404 studies, Supporting Information Table S2). N-fixing species
360 are present in reference area plant communities in 58 studies and of those studies, 20 studies
361 include an invasive species that is also an N-fixing species (Supporting Information Table S2). A
362 study's N-fixing status is important for explaining three soil nitrogen invasion effect sizes: total
363 inorganic N, soil N, and soil C : N (Fig. 2). For total inorganic N, studies with only resident N-
364 fixers have significantly lower and negative effect sizes than studies without resident N-fixers. In
365 fact, studies with N-fixers only in the reference plant community are the only studies to show a
366 decrease in total inorganic N in invaded plots. For soil N, studies with only invasive N-fixers

367 tend to have larger soil N effect sizes, but post-hoc tests reveal that the only levels that differ are
368 studies with invasive species N-fixers only and studies without any N-fixers (Fig. 2). For soil C :
369 N, studies with only resident N-fixers have significantly higher soil C : N effect sizes than the
370 other N-fixing status levels and higher soil C : N values in invaded than reference areas.

371

372 *Study design*

373 Observational field studies are by far the most frequent type of study in this dataset (273 of 404
374 studies, Supporting Information Table S2). Study design is a significant predictor for nitrate and
375 soil organic matter effect sizes (Fig. 3). For nitrate effect sizes, all study designs except
376 observational field studies exhibit neutral or slightly negative effect sizes, whereas observational
377 field studies found significantly higher nitrate pools in invaded areas relative to reference areas
378 (Fig. 3). Soil organic matter values were marginally to significantly higher in invaded areas for
379 studies that are experimental removals and greenhouse experiment study designs. Soil organic
380 matter effect sizes in observational studies do not significantly differ based on invasion (Fig. 3).
381 Only one experimental addition study measured soil organic matter.

382

383

384 **Discussion**

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

398 and to quantifying reference or “baseline” community traits in areas that are vulnerable to
399 invasion.

400

401 *Global effect sizes*

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

417

418 *Invasive species’ leaf and litter traits*

419 When apparent, the associations we detected between invader traits and impacts fit our
420 predictions. Greater invasion impacts were observed when the invasive species had N-rich trait
421 values (e.g. high N content and low C : N), whereas more modest shifts in N cycling occurred
422 where the reference community had N-rich CWM trait values. Only one invasive species’ plant
423 trait (leaf N content) explained variation in the magnitude and direction of changes in soil N due
424 to invasion. In this case where an invader trait could explain invasion effect sizes on a soil
425 property, our hypotheses regarding the direction of the relationship were supported. The varying
426 explanatory power of different traits for different effect sizes is not surprising given the variation
427 in data coverage among trait types. The trait that was most widely reported in the literature,
428 invasive species’ leaf N content, explained variation in bulk soil properties like soil N and soil C

429 : N. It is impossible to tell whether our inability to detect the effects of other plant traits on N
430 cycling is a result of the small sample size or an actual lack of effects. Variability in the extent to
431 which these traits are informative could have biological significance since particular traits may
432 be more or less integral to the ecosystem function of interest (Díaz *et al.*, 2004), and the
433 particular traits that are integral for a species in a given environment may not be the same traits
434 that are integral in the context of a different invasion. For example, *Bromus tectorum*'s leaf N
435 content may be a more important driver of its impact on soil inorganic N than that of *Morella*
436 (*Myrica*) *faya*, since *M. faya*'s status as an N-fixing species is more closely associated with its
437 soil N impacts. In addition, some of the ways in which invasive plant species alter soil N cycling
438 may not have been well characterized by the traits included in this study. Belowground plant
439 traits (e.g. mycorrhizal association type or root exudation rate) may have more explanatory
440 power for some invasive species and contexts (Bardgett *et al.*, 2014), but are more rarely
441 measured or reported. And finally, another reason that some traits and effect sizes may not be
442 strongly correlated is because soil N processes are also regulated by abiotic factors such as soil
443 type, moisture, and temperature which can obscure expected relationships between plant
444 nutrient-use traits and soil N values (Castro-Díez *et al.*, 2014; Hobbie, 2015).

445

446 *Reference community leaf and litter traits*

447 There is limited evidence that reference community characteristics besides N-fixing status shape
448 invasion impacts on N cycling. As previous studies have found, invasion impacts on N cycling
449 are diminished where N-fixing species are already present (Vilà *et al.*, 2011; Castro-Díez *et al.*,
450 2014). However, we did not find that reference CWMs alone were very strong predictors of
451 invader effects on N cycling. Reference species traits did not contain sufficient information to
452 explain invasion effects on soil N pools or fluxes. Overall, reference community plant tissue
453 traits do not perform well at explaining variability in invasion impacts, and this might be because
454 reference plant communities can shape N cycling through many other pathways, including by
455 altering abiotic conditions that mediate N transformations.

456

457 *Trait dissimilarities*

458 Dissimilarity in leaf and litter traits among invaded and reference communities explained the
459 magnitude and direction of the most types of invasion impacts on soil N (i.e. pools, fluxes, and

460 bulk soil properties). Trait dissimilarities were able to capture most of the trends detected by
461 invasive species' traits and reference community traits, while in addition, explaining variation in
462 inorganic N flux effect sizes. This result supports the idea, identified by previous reviewers
463 (Ehrenfeld, 2003; Castro-Díez *et al.*, 2014), that invasive species can have both direct and
464 indirect impacts on N cycling by having dissimilar characteristics and potentially by displacing
465 native plant species that are likewise important in shaping N pools and fluxes. To calculate the
466 changes in plant trait values that result from invasion, we used CWM trait estimates from the
467 invaded and reference areas so as to reflect the way that invasion impacts on ecosystem
468 processes are calculated (Garnier *et al.*, 2004; Díaz *et al.*, 2007). Some field studies use CWM
469 traits to explain variation in soil N cycling in invaded and reference areas, but until now, there
470 has never been a global-scale synthesis to evaluate the explanatory power of this information.
471 Our findings suggest that dissimilarity between how invaded and reference plant communities
472 take up and release nutrients, approximated by leaf and litter traits, is critical to understanding
473 variability in invasion impacts on N cycling. Whereas leaf N content was the most informative
474 invasive species' traits, the dissimilarity in C : N ratio between invaded and reference
475 communities better explained the impact of invasion on soil N pools and fluxes. This finding
476 could suggest that plant tissue stoichiometry is a more important indicator of ecosystem
477 sensitivity to soil N cycling perturbations than plant tissue N content alone.

478

479 *Study design*

480 On the whole, the influence of study design on the magnitude and direction of invasion effect
481 sizes was limited with only 2 of 9 effect sizes mediated by study design. This fits with the
482 findings of Castro-Díez *et al.* (2014) in that study design does not appear to strongly mediate
483 detection of invasion impacts on soil N cycling. But it is important to note that both Castro-Díez
484 *et al.* (2014) and this study detected a few ways that soil N cycling measures were sensitive to
485 study design. We found that invasions are associated with larger soil nitrate pools in
486 observational studies but not experimental studies, whereas Castro-Díez *et al.* (2014) found no
487 difference in invasion effect sizes on soil N pools and fluxes based on whether the study was
488 observational, experimental, or a combination. Our finding might suggest that the impact of
489 invasion on nitrate pools develops only after a long lag period since observational studies are
490 more likely to capture an older invasion than manipulative experiments. Another possibility, not

491 mutually exclusive from the previous one, is that high nitrate concentrations promote invasion
492 and are not a consequence of invasion. In contrast to Castro-Díez *et al.* (2014)'s finding that
493 removal studies have smaller N pool and flux effect sizes than studies with a non-invaded
494 reference, we found that soil organic matter effect size was the only impact type that was
495 sensitive to experimental removal studies. We found that invaded areas are associated with more
496 soil organic matter in experimental removal and greenhouse studies than observational studies.
497 This finding is supported by only a few studies, but may suggest that experimental removal
498 disrupts soil organic matter whereas greenhouse studies involve extensive root proliferation in
499 invaded pots that is difficult to separate from the soil matrix. Alternatively, we may only be able
500 to detect the impact of invasion on soil organic matter in highly controlled studies such as field
501 and greenhouse experiments. More work is needed to understand why invasion appears to
502 increase soil organic matter in only these study design types. A variety of study designs are
503 needed to understand how invasions influence and are influenced by nitrate and soil organic
504 matter, but on the whole, researchers should be pleasantly surprised to know that observational
505 studies do provide similar insights to invasion impacts as experimental studies.

506

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

516

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532

533 **Author Contributions**

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

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713 **Tables**

714

715 **Table 1.** Invasion tends to promote larger and faster soil N pools and fluxes based on four meta-analyses, including this study.

716 Invasion effect size values for 9 measurements that characterize soil inorganic N pools, fluxes, soil N, C : N and organic matter; mean

717 +/- 95% confidence interval with the number of studies, k. All effect size values are calculated as Hedges' d (d⁺), except Liao *et al.*

718 2008, which is calculated as a weighted response ratio (RR₊₊). Grey highlight indicates that the 95% confidence interval does not

719 overlap zero. Effect sizes >0 indicate that measurement values are greater in invaded than reference areas.

Soil measurement		This study			Liao <i>et al.</i> 2008			Vila <i>et al.</i> 2011			Castro-Diez <i>et al.</i> 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

720

721

722 **Table 2.** Summary of mixed effects models used to explain invasion effect sizes (response variable) using invasive species' trait
723 values, reference community-weighted mean (CWM) trait values, or dissimilarity between invaded and reference CWMs as fixed
724 effects. Random effects account for variation within studies and among studies within the same article. For each model fit, the
725 following data are reported: hypothesized relationship direction (Hyp.), fixed-effect coefficient (est), pseudo-R² (R_p²), and number of
726 studies (k). Models with a significant fixed effect after Holm-Bonferroni corrections for multiple comparisons are highlighted in bold
727 grey.

Response variable		Trait type	Hyp.	Invasive species						Reference CWM						CWM Dissimilarity							
				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	0.00	122	0.56	0.00	19	-	-0.14	0.00	117	-0.11	0.00	26	+	0.19	0.00	115	0.61	0.00	26
	NO ₃ ⁻		+	0.07	0.00	128	-0.01	0.00	20	-	-0.04	0.00	124	0.01	0.00	30	+	0.11	0.00	122	0.18	0.00	30
	NH ₄ ⁺ +NO ₃ ⁻		+	0.09	0.00	173	0.51	0.00	21	-	-0.06	0.00	146	0.17	0.00	24	+	0.15	0.00	144	1.55	0.72	24
N flux	Δ NH ₄ ⁺		+	0.12	0.00	38	0.86	0.00	13	-	0.05	0.00	38	-0.53	0.00	15	+	-0.06	0.00	38	0.32	0.00	15
	Δ NO ₃ ⁻		+	0.14	0.00	67	0.81	0.80	16	-	0.13	0.00	65	-1.23	0.51	18	+	0.19	0.00	65	0.58	0.62	18
	Δ NH ₄ ⁺ +NO ₃ ⁻		+	0.01	0.00	84	0.04	1.33	15	-	-0.01	0.00	77	-0.04	0.43	18	+	0.21	0.00	77	-0.10	0.48	18
Other	Soil N		+	0.34	0.09	170	0.51	0.47	27	-	0.16	0.00	154	-0.04	0.00	28	+	-0.03	0.00	152	0.34	0.00	28
	Soil C:N		-	-0.20	0.00	89	-0.46	0.03	10	+	-0.05	0.00	81	0.27	0.00	12	-	0.01	0.00	81	-0.38	0.09	12
	SOM		+	0.27	0.71	88	0.11	2.32	19	-	0.15	0.86	81	-0.36	2.11	18	+	-0.03	0.82	81	0.51	2.11	18
Inorganic N pool	NH ₄ ⁺	C:N	-	-0.02	0.00	64	-0.02	0.00	11	+	0.01	0.00	48	0.01	0.00	17	-	-0.01	0.00	48	-0.02	0.00	17
	NO ₃ ⁻		-	0.00	0.00	75	-0.03	0.00	12	+	0.01	0.00	61	0.00	0.00	18	-	0.00	0.00	61	-0.02	0.00	18
	NH ₄ ⁺ +NO ₃ ⁻		-	-0.01	0.00	97	-0.01	0.00	14	+	0.01	0.00	70	0.01	0.00	23	-	-0.02	0.00	70	-0.02	0.35	23
N flux	Δ NH ₄ ⁺		-	0.01	0.01	19	-0.01	0.00	7	+	0.01	0.02	16	0.00	0.00	10	-	-0.01	0.00	16	0.00	0.00	10
	Δ NO ₃ ⁻		-	0.00	0.00	38	-0.01	0.00	14	+	0.00	0.00	28	0.01	0.00	18	-	0.00	0.00	28	-0.01	0.00	18
	Δ NH ₄ ⁺ +NO ₃ ⁻		-	0.00	0.00	59	-0.01	0.00	16	+	0.02	0.00	46	0.00	0.00	22	-	-0.03	0.00	46	-0.01	0.00	22
Other	Soil N		-	-0.01	0.00	101	-0.02	0.00	20	+	0.00	0.00	89	0.01	0.00	23	-	-0.01	0.00	89	-0.01	0.06	23
	Soil C:N		+	0.00	0.00	70	NA	NA	12	-	-0.02	0.00	66	0.00	0.00	13	+	0.03	0.00	66	0.01	0.00	13
	SOM		-	0.03	0.00	28	-0.03	0.34	13	+	0.02	0.00	22	0.00	0.00	13	-	-0.01	0.00	22	0.00	0.00	13

728 NA indicates that the model did not converge

729

730 **Figure Legends**

731

732 **Figure 1.** Community-weighted mean (CWM) trait dissimilarities between invaded and
733 reference plant communities shape the magnitude and direction of invasion impacts on aspects of
734 soil N cycling. **(a)** Greater litter N content and **(b)** lower litter C : N in invaded than reference
735 communities is associated with larger increases in total inorganic N due to invasion. Greater leaf
736 C : N in invaded than reference communities is associated with **(c)** smaller increases and even
737 decreases in mineralization and **(d)** smaller declines and even increases in soil C : N. Point size is
738 scaled by study variance such that larger points have smaller variance. Point shade represents
739 CWM quality rank such that darker points are higher in quality. Trait dissimilarity is a significant
740 fixed effect in all regression models shown. Solid line and blue shaded region show the meta-
741 regression model fit and the 95% confidence interval; see Table 2 for fit statistics. Dashed $y=0$
742 line aids in distinguishing between studies with positive (higher values in invaded area) and
743 negative (lower values in invaded area) effect sizes.

744

745 **Figure 2.** Presence of N-fixing plant species shapes the magnitude and direction of invasion
746 impacts on total inorganic N, soil N, and soil C : N effect sizes. Different letters indicates that
747 levels are significantly different according to post-hoc t-tests ($\alpha=.05$). Effect sizes were
748 calculated as the standard mean difference (Hedges' d) of invaded minus reference areas such
749 that values >0 indicate greater values in invaded than reference areas; mean \pm 95% confidence
750 interval with the number of studies shown in parentheses.

751

752 **Figure 3.** Study design mediates soil nitrate and organic matter invasion effect sizes. Different
753 letters indicate significant differences among study designs according to post-hoc t-tests
754 ($\alpha=.05$). Effect sizes were calculated as the standard mean difference (Hedges' d) of invaded
755 minus reference areas such that values >0 indicate greater values in invaded than reference areas;
756 mean \pm 95% confidence interval with the number of studies shown in parentheses.

757 **Supporting Information Legends**

758

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

760

761 **Figure S2** Frequency distribution of invasive species' trait values and CWM trait dissimilarities.

762

763 **Figure S3** Effect size funnel plots.

764

765 **Figure S4** Invasive species' with higher leaf %N promote greater increases in soil N.

766

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

769

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

772

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

774

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

777

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

782

783 **Methods S1** Hedges' d calculation.

784

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

786

787 **Methods S3** Pseudo-R² calculation.

788

789 **Notes S1** Invasion impacts on soil nitrogen cycling references.