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

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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.

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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

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

5

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*

139   *al.*, 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.

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147

148   **Materials and Methods**

149

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; Schrodt *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).

316

317

318 **Results**

319

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).

327

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±0.1% leaf N, 1.2±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±0.0% leaf N,

342   1.0±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±0.04% leaf N and +0.19±0.07% litter N, +0.84±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

14

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

17

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

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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712

Tables

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 *et al.* 2008			Vila *et al.* 2011			Castro-Diez *et al.* 2014		
		Effect size (d^+)	95% CI	k	Effect size (RR_{++})	95% CI	k	Effect size (d^+)	95% CI	k	Effect size (d^+)	95% CI	k
Inorganic N pool	NH_4^+	0.26	0.12 to 0.40	141	0.26	0.23 to 0.29	87						
	NO_3^-	0.17	-0.003 to 0.34	150	0.15	0.12 to 0.18	77						
	$NH_4^+ + NO_3^-$	0.15	-0.05 to 0.35	195				0.63	0.29 to 0.96	47	0.28	-0.06 to 0.62	45
N flux	ΔNH_4^+	-0.05	-0.31 to 0.20	50									
	ΔNO_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			
	$\Delta NH_4^+ + NO_3^-$	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-R^2 (R_p^2), 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						Hyp.	Reference CWM						Hyp.	CWM Dissimilarity					
				Leaf			Litter				Leaf			Litter				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	ΔNH_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
	ΔNO_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
	$\Delta NH_4^+ + NO_3^-$		+	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	ΔNH_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
	ΔNO_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
	$\Delta NH_4^+ + NO_3^-$		-	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

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

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-R^2 calculation.

29

788

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