1 Title: **The relative importance for plant invasiveness of trait means, and**

2 **their plasticity and integration in a multivariate framework**

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Summary

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28   · Functional traits, their plasticity and their integration in a phenotype have
29   profound impacts on plant performance. We developed structural equation models
30   (SEM) to evaluate their relative contribution to promote invasiveness in plants
31   along resource gradients.

32   · We compared 20 invasive-native phylogenetically and ecologically related pairs.
33   SEM included one morphological (Root/Shoot ratio, R/S) and one physiological
34   (Photosynthesis Nitrogen Use Efficiency, PNUE) trait, their plasticities in
35   response to nutrient and light variation, and phenotypic integration among 31
36   traits. Additionally, these components were related to two fitness estimators,
37   biomass and survival.

38   · The relative contribution of traits, plasticity and integration was similar between
39   invasive and native species. Traits means were more important than plasticity and
40   integration for fitness. Invasive species showed higher fitness than natives
41   because i) they had lower R/S and higher PNUE values across gradients, ii) their
42   higher PNUE plasticity positively influenced biomass and thus survival, and iii)
43   they offset more the cases where plasticity and integration had a negative direct
44   effect on fitness.

45   · Our results suggest that invasiveness is promoted by higher values in the fitness
46   hierarchy, traits means are more important than trait plasticity, and plasticity is
47   similar to integration, rather than by a specific combination of the three
48   components of the functional strategy.

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Introduction

Many different factors may determine plant invasiveness. At the level of particular functional traits, high phenotypic plasticity and high phenotypic integration have been hypothesized as potential factors promoting invasion success (Pigliucci & Preston, 2004; Hamilton *et al.*, 2005; Richards *et al.*, 2006). After decades of research, information about plant traits associated with invasiveness is clear. Exhaustive reviews (Daehler, 2003; Pyšek & Richardson, 2007) and meta-analysis (van Kleunen *et al.*, 2010) have shown that specific traits related to physiology, morphology, biomass allocation, growth rate, and size differ between invasive and non-invasive/native species. For instance, high maximum photosynthetic rate, high SLA, low root/shoot ratio, high fecundity, high relative growth rate and high reproductive effort are usually associated with invasiveness.

Research on phenotypic plasticity has been less exhaustive and current empirical studies give mixed results. Theoretically, high phenotypic plasticity may promote invasiveness because it helps exotic species express advantageous phenotypes over a broad range of environments (Gray, 1986; Williams *et al.*, 1995; Alpert *et al.*, 2000; Daehler, 2003; Matesanz *et al.*, 2010). However, while several empirical studies illustrate this hypothesis, others do not (Funk, 2008; Schumacher *et al.*, 2009; Godoy *et al.,* 2011; Palacio-López & Gianoli, 2011). Mismatches between studies may be due to the fact that observed plasticity is assumed to be adaptive, even though the positive relationship between plasticity and fitness is not ubiquitous and must be explicitly demonstrated (Baker, 1965; Sultan, 2001; Richards *et al.*, 2006; Hulme, 2008). An increase in plasticity may not increase fitness (non-adaptive plasticity) and may even decrease it (maladaptive plasticity; e.g. van Kleunen & Fischer, 2005; Valladares *et al.*, 2007).

Finally, phenotypic integration, defined as the pattern of functional correlation among different plant traits (Pigliucci, 2003), may act as an important feature conferring invasiveness. For instance, an integrated phenotype may respond to environmental variation more efficiently, producing a more adaptive response to the environment than less integrated phenotypes (Schlichting, 1989; Waitt & Levin, 1993; Gianoli, 2004). In

82   addition, phenotypic integration may increase survival by reducing the cost of

83   maladaptive and/or non-adaptive plastic traits (van Kleunen & Fischer, 2005; Poot &

84   Lambers, 2008). However, our empirical knowledge about phenotypic integration is

85   scarce and more work is needed for a better understanding of the role of phenotypic

86   integration in plants fitness (Pigliucci & Preston, 2004). For instance, previous empirical

87   studies found a negative relationship between phenotypic plasticity and phenotypic

88   integration (Gianoli, 2004; Gianoli & Palacio-López, 2009), a surprising finding because

89   theoretically both can favour plant fitness.

90   Rather than continuing to study aside whether particular traits, their plasticity or

91   their integration are linked to invasiveness, it is more relevant to have a solid knowledge

92   about how these three aspects of the plant strategy promote plant fitness. To promote a

93   rapid exclusion, exotic species must show high fitness differences with native species

94   (Macdougall *et al.,* 2009). This may be achieved by adequately responding to fluctuations

95   in a given resource, but also by avoiding potential future costs resulting from non-

96   adaptive responses (van Kleunen & Fischer, 2005; Valladares *et al.*, 2007). For instance,

97   high fitness differences due to adaptive plastic responses may be more likely to occur in a

98   less constrained phenotype (i.e. with low phenotypic integration), or in a highly stressful

99   environment (Richards *et al.*, 2006). Moreover, superior performance may be

100   underpinned by a combination of higher mean trait and greater adaptive plasticity

101   (Godoy *et al.,* 2011).

102   In a previous study comparing twenty invasive-native phylogenetically and

103   ecologically related plant pairs, we observed that invasive species showed higher biomass

104   gain and survival after six months of growth than native species (Godoy *et al.,* 2011).

105   Here, our aims are to unravel 1) how trait means, phenotypic plasticity and phenotypic

106   integration contribute to fitness differences between invasive and native species, and 2)

107   how the relative importance of these three components of the functional strategy vary

108   along resource gradients. These aims were tackled in a multivariate way by means of

109   Structural Equation Modelling (SEM) (see Shipley, 1999; Shipley, 2002; Shipley, 2004

110   for details).

111   Our aprioristic model posits that both key morphological and physiological traits,

112   and their corresponding plasticities, directly affect fitness (as found by Daehler, 2003;

113   Funk, 2008). We assume that biomass influences the survival rate, and both are critical

114   surrogates of fitness for perennial plants or in short term studies (Sultan, 2001). We

115   expect a negative correlation between phenotypic integration and morphological and

116   physiological plasticities (Gianoli, 2004; Gianoli & Palacio-López, 2009). Thus,

117   phenotypic integration may increase biomass and survival as opposed to trait plasticity

118   (see Fig. 1A for SEM structure). We hypothesize that invasive species will show higher

119   fitness because the overall contribution of their trait means and trait plasticity is higher.

120   We also hypothesize that higher adaptive trait plasticity will contribute more to the

121   invasive species fitness under resource-limited conditions, while particular trait values

122   will be more relevant for invasive species to achieve fitness in non-limiting parts of

123   resource gradients (e.g. Funk 2008). Finally, we may also expect that phenotypic

124   integration have a positive effect on the increment of biomass and survival, from limiting

125   to non-limiting conditions (Schlichting, 1989; Waitt & Levin, 1993; Gianoli, 2004).

126   However, we do not have any previous support to hypothesize that this positive effect

127   will be higher in invasive species.

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129   **Material and Methods**

130   *Species selection and experimental set-up*

131   We   selected   twenty   exotic   species   clearly   invasive   in   the Iberian

132   Peninsula (*sensu* Pyšek *et al.*, 2004), as they are local dominants in some native

133   ecosystems (Valéry *et al.*, 2008), and have a potential impact on the native ecosystems

134   (transformer species, *sensu* Richardson *et al.*, 2000) (Table 1). They represent a broad

135   range of taxonomic groups, habitat preferences (woodlands, shrublands, grasslands and

136   riparian areas) and growth forms (annual and biannual herbs, shrubs and trees). Then, we

137   paired each invasive species with one closely related native species of the Iberian

138   Peninsula based on a suite of phylogenetic and ecological criteria: a) the native species

139   had to belong to the same family as the invasive species, which was achieved in 17 of the

140   20 pairs, b) they had to have the same growth form (achieved in all pairs except number

141   15 and 16, in which invasive species were trees and natives shrubs), c) they had to co-

142   exist in the same habitat-type in the Iberian Peninsula and the same successional

143   community stage, and d) they had to be recorded as co-occurring at least once in Spain

144   (Table 1). We consulted the extensive Herbarium database at Universidad Complutense

145   de Madrid (MACB, founded 1968) to check for co-occurrence within pairs. Native

146   species with small distribution ranges, rare or with endangered status were excluded.

147   Moreover, only three of our 20 native species selected were recorded invasive elsewhere.

148   So our native species set can be mostly considered as non-invasive as well.

149   We designed a non-factorial experiment with two different resource

150   gradients: nutrient gradient with three levels (Low-Medium-High) and light gradient

151   with two levels (Shade-Sun). In the nutrient gradient, low level was equivalent to 0.010g

152   N, medium level to 0.085g N and high level to 0.245g N per plant. In the light gradient

153   shade level was equivalent to 20% full radiation (max PPFD 350-500 $\mu molm^{-2}s^{-1}$) with

154   light quality modified to Red/far Red=0.8 (to mimic natural shade effects on the light

155   spectrum by establishing layers of green cloth), and sun level was equivalent to 50% full

156   radiation (max PPFD 950-1050 $\mu molm^{-2}s^{-1}$) with no Red/far Red modification. We

157   avoided a 100% light intensity for sun level because the high irradiance of the

158   experimental site during the summer could compromise the viability of the experiment. In

159   the nutrient gradient, light was kept constant at 50% full radiation (950-1050 $\mu molm^{-2}s^{-1}$)

160   and in the light gradient N doses were kept constant at medium nitrogen level (0.085g N

161   per plant). This combination of factors represents *a priori* a change in the resource

162 availability from limiting to non-limiting (low to medium nutrient level, and shade to sun

163 light) to two levels of non-limiting resources (medium to high nutrient level).

164   Each species per resource level was replicated three times (blocks) to control for

165 possible micro-environmental variations and each block contained 12 individuals per

166 species. In total, we used a total of 5760 plants, 144 per species (40 species x 4 treatments

167 x 3 blocks x 12 plants each block). Plants were grown from seeds in 1 L pots (QP 12T/18,

168 PROJAR, Spain) from February to September in each of the two years (2005-2006) that

169 the study was carried on. Seeds were obtained from commercial supply or field

170 collection. In both cases, seeds came from locations where the exotic species are clearly

171 invasive. From commercial supply, seeds were certified to come from one single location.

172 From field collection, we collected seeds from 15-20 haphazardly chosen plants within

173 one population. Population delimitation followed similar procedure to other works such

174 as Schlaepfer *et al.*, (2010) (i.e. one population was defined as continuous stands of

175 species covering an area between 4 and 40 000 m^2 and separate from other populations at

176 least 10km, Appendix S1 for locations). Just after seed germination, we fertilized

177 seedlings with a Plantacote mix 6 month slow-release fertilizer 14-9-15 N-P-K, (Aglukon

178 Spezialdünger GMBH & Co.KG, Dusseldorf, Germany). We used a slow-release

179 fertilizer to ensure that plants had available nitrogen throughout the experiment. The main

180 nitrogen compound was ammonium nitrate (NH_4NO_3) (85%). Pure vermiculite was used

181 as substrate to ensure that the fertilizer was the only source of nutrient supply. The

182 gravimetric soil water content in the pots was maintained at >30%. Local air temperature

183 and available photosynthetic photon flux density (PPFD) were recorded every 5 min

184 throughout the growing season with a data logger (HOBO model H08- 006-04; Onset,

185 Pocasset, MA, USA) and self-made external sensors that were cross-calibrated with a Li-

186 Cor 190SA sensor (Li-Cor, Lincoln, NB, USA). Mean daily temperature was 17.3ºC

187 (ranging from 9.6ºC to 22.5ºC) and mean daily PPFD (400–700 nm) over the summer

188 was 41 mol m^2d^{-1}, which is equivalent to full sunlight.

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Phenotypic traits, plasticity and integration

For each species and treatment, we measured 31 traits related to canopy structure, allometry and leaf physiology characteristics as well as total biomass and survival at the end of the experiment (Table 2 and Table S1 for mean ± SE values for each trait, species, and treatment). A large number of traits were selected mainly for two reasons. First, to have an ample variety of suitable traits among which the most relevant in this study would be included into the SEM analyses (see below *Structural equation modeling of phenotypic performance* section), and second, to support phenotypic integration data by covering multiple aspects of trait functionality at different plant-scales. Thus, traits were selected because of their functional significance for resource acquisition (e.g. high LAR and RWR are associated with light and nutrient acquisition, respectively), plant competition (e.g. high rate of maximum photosynthesis and Fv/Fm are associated with fast growth and optimal physiological state), and stress tolerance (e.g. high PNUE is associated with high plant performance in nitrogen limited environments and high SLA in light limited environments). Most of these traits have been previously included in studies and meta-analyses comparing invasive vs. non-invasive/native species due to their importance for plant performance (Sultan, 2001; Funk, 2008; van Kleunen *et al*,. 2010), and because they are known to respond to light and nutrient gradients (Valladares *et al.*, 2000; Funk, 2008; Schumacher *et al.*, 2009).

We calculated phenotypic plasticity of each trait and species using the relative distance plasticity index (RDPI) (Valladares *et al.,* 2006). Prior to any analysis, trait data was log-transformed (log[x]) to avoid differences in scale within and between traits. We used RPDI because 1) it is highly correlated with other indices of phenotypic plasticity commonly used in the literature (Valladares *et al.,* 2006), and 2) it has the advantage to be the only index that provide a statistical distribution of relative distances which can be implemented into SEM analyses (see below *Structural equation modelling of phenotypic*

216 *performance* section) and phenotypic integration estimations (see below). The number of

217 relative distances was equal to the number of replicates per treatment to avoid

218 pseduoreplication. Each relative distance was calculated as the absolute value of the trait

219 distance between two randomly selected individuals of the same species belonging to two

220 different environments, divided by the sum of their trait values.

221 Additionally, we obtained an overall standardized RDPI value, one per species

222 and trait, ranging from 0 (no plasticity) to 1 (maximum plasticity) by summing all

223 relatives distances obtained and dividing by the total number of distances (Valladares *et*

224 *al.,* 2006). These standardized RDPI values were used to test for differences in trait

225 plasticity between species (see below PERMANOVA analyses). For the nutrient

226 treatment, we calculated *RDPI* from low to medium nutrient level and medium to high

227 nutrient level. For the light treatment, *RDPI* was calculated from shade to sun.

228 We defined phenotypic integration as the joint variation of two different traits in

229 response to an environmental change. We estimated phenotypic integration at the level of

230 species as the percentage of significant correlated plastic responses among traits between

231 two treatments (i.e. low to medium nutrient, medium to high nutrient, shade to sun)

232 (Schlichting 1989; Schlichting & Pigliucci 1998). Thus, phenotypic integration varies

233 from 0 (no integration) to 1 (full integration). The number of plasticity replicates (i.e.

234 number of relative distances obtained with RDPI) for estimating whether plastic

235 responses among two traits were correlated, varied from 3 to 9 for each species and

236 treatment depending on the trait measured (Appendix S2).

237 To our knowledge, this is the first study that estimates phenotypic integration in

238 such a high number of traits and this may impose a methodological trade-off. On one

239 hand, this has the advantage of increasing the likelihood that traits accounting for

240 phenotypic integration will be included. On the other hand, this has the disadvantage of

241 inflating the total number of potential correlations, and hence we might underestimate the

242 value of phenotypic integration. To handle this methodological trade-off, we constrained

243   the set of correlations to major axes of variation with functional meaning and then, we

244   averaged the percentage of correlated plastic responses for these axes to obtain an overall

245   estimation of phenotypic integration per species.

246   We obtained three major axes of functional variation by performing a principal

247   component Analysis (PCA) (see PCA loadings, Table S2). The first axis (PC1) can be

248   interpreted as a measure of physiological processes occurring on the leaves at a molecular

249   and electronic level. It was positively related to photosynthetic machinery traits (e.g.

250   A_{max}, qP reflect chlorophyll pigments) and negatively related to traits reflecting high

251   contents of photoprotective pigments (e.g. qN and NPQ reflect xanthophylls pigments).

252   The second axis (PC2) grouped the role that leaf area and leaf nitrogen content play at

253   different plant scales. This axis described important functional trade-offs such as the

254   negative correlation between SLA and N_{area} found for the leaf economics spectrum

255   (Wright *et al*., 2004) and the negative correlation between water use efficiency (iWUE)

256   and leaf area allometry (SLA, LAR) (Reich *et al*., 1989, Poorter *et al*., 1990). The third

257   axis (PC3) accounted for the weight that roots have on the total plant biomass and

258   described the trade-off between below- and above-ground growths (Weiner, 2004).

259

260   *Statistical analysis testing for differences in fitness, phenotypic traits, plasticity and*

261   *integration*

262   We performed PERMANOVA analyses to test for differences between invasive and

263   natives in: 1) fitness estimators (biomass and survival), 2) R/S and PNUE means, 3) R/S

264   and PNUE plasticity, and 4) phenotypic integration. We selected PERMANOVA

265   approach because it permits pairwise comparison at different phylogenetic levels in

266   agreement with our experimental design and also because we could not always reach the

267   assumptions of normality and homocedasticity of the data and its residuals (Anderson,

268   2001, Anderson, 2005). We performed an analysis for each variable considering

269   invasive/native status and nutrient/light levels as fixed-factor, block as a random-factor

270 and phylogenetic distance within pairs as a co-variable. Analyses were conducted within

271 treatments (R/S and PNUE) and between treatments (R/S plasticity, PNUE plasticity and

272 phenotypic integration). Additionally, total biomass was also included as a covariate

273 when analyzing differences in R/S and PNUE plasticity to check whether plastic

274 responses was a mechanistic consequence of an increase in plant size (i.e. apparent

275 plasticity) (Dudley 2004, Funk 2008). In all cases, differences between both groups and

276 post hoc comparisons were estimated using the Bray-Curtis dissimilarity distance from

277 9999 permutations. The phylogenetic distance from one species to another for each of the

278 species pairs was calculated through to the first common ancestor to both species using

279 the plant phylogenetic supertree described by Soltis *et al.*, (2000) and modifications by

280 Bremer *et al.*, (2003).

281

282 *Structural equation modelling of phenotypic performance*

283 SEM provides an aprioristic-statistical approach that can be used to unravel the linking

284 structure of traits that are correlated in a multivariate way based on previous knowledge

285 (Shipley, 2004). We used SEM (1) to investigate the relative contribution of mean trait

286 values, phenotypic plasticity, and phenotypic integration to fitness differences between

287 invasive and native species along resource variation, and (2) to disentangle direct from

288 indirect effects of the three properties on fitness. The overall causal structure relating

289 these components of the functional strategy were previously introduced (Fig. 1A).

290   We selected Root/Shoot ratio (R/S) and Photosynthetic Nitrogen Use Efficiency

291 (PNUE) as the key morphological and the physiological traits to predict plant fitness

292 because of their importance in competition and stress-tolerance and because they showed
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295   allometric plastic responses to resource variation (Weiner 2004) (Figure S1). R/S ratio

296   was highly correlated to Leaf Area Ratio (LAR) (r>0.80, d.f.=479) and R/S and LAR

297   plasticities were correlated too (p-value<0.01) (from low to medium nutrient (L-M),

298   r=0.76 from medium to high nutrient (M-H), r=0.67 from shade to sun light (SH-S)

299   r=0.82, d.f.=119). The capacity to capture soil resources is dependent on R/S (Hodge,

300   2004) and the ability to capture light for photosynthesis is related to their LAR

301   (Valladares *et al.*, 2002). High plasticity of R/S and LAR are often considered as a

302   strategy to maximize the capture of limiting resources, such as light, nutrient or water

303   (Valladares *et al.*, 2002; Poot & Lambers, 2008). PNUE integrates nitrogen leaf

304   concentration (N_{mass}) and maximum photosynthetic rate (A_{max}). Thus, it was highly

305   correlated to both physiological traits and their plasticities were correlated too. PNUE

306   also correlates with Specific Leaf Area (SLA) (Poorter & Evans, 1998) as occurred in our

307   experiment (r=0.73, p<0.05, d.f.=479). PNUE provides insight on the efficiency of

308   photosynthetic machinery (Poorter & Evans, 1998) and its plasticity is highly related to

309   the competitive ability of plants (Funk, 2008).

310   To address the fact that we needed to analyze traits values (obtained within a level

311   of resources) with plasticity and integration values (obtained between two levels), we

312   fitted the model with the trait values of the level with higher resources. For instance, we

313   used R/S and PNUE values from the medium nutrient level when SEM model was

314   performed from low to medium nutrient level, and the same procedure was done from

315   medium to high nutrient level and from shade to sun. Biomass in the high resource level

316   and differences of biomass between resource levels were highly correlated across

317   treatments (r>0.85, p<0.001, d.f.=359); hence, increase in biomass between treatment

318   levels was included in the SEM models (Fig. 1A).

319   We assessed whether our aprioristic SEM fit the data by a series of goodness-of-

320   fit tests, which compared the observed covariance matrix to that derived from the model

321   (Shipley, 2002). First, we performed a χ^2 test to evaluate the goodness-of-fit of our

322   model. However, given that our data not always adjusted to a multinormal distribution,

323   we performed other goodness-of-fit tests, such as the goodness-of-fit index (GFI) and

324   Bentler's-Bonett's normed-fit index (NFI) (Shipley, 2002; Iriondo *et al.*, 2003). GFI and

325   NFI range between 0 and 1, with values >0.90 indicating a good fit. For R/S and PNUE

326   and R/S plasticity and PNUE plasticity, a total of 360 values were included (3 replicates

327   per block x 3 blocks x 40 species). For phenotypic integration, only 40 values (40

328   species) were included because replicates of individuals and blocks were used to estimate

329   the percentage of correlated plastic responses among traits per species. Then, we used the

330   generalized least-squares (GLS) method to estimate the standardized path coefficients of

331   our model, which are equivalent to standardized partial regression coefficients (i.e. they

332   define the relative influence of one variable on another), and its significance with

333   multivariate Wald test. This test locates the set of path coefficients that can be considered

334   zero without worsening the fit (i.e., significantly increasing the χ^2) of the model (Shipley,

335   2002).

336   Additionally, we performed explicit comparisons between invasive and native

337   species through multigroup analysis (Shipley, 2002; Byrne, 2004; Milla, 2009) because

338   we aim to distinguish whether the paths of the model statistically differ between invasive

339   and native species. The statistical procedure was first to build a constrained model, in

340   which all free parameters were forced to be equal across invasive and native species. This

341   model was then compared with the outcome of the model fitted to the experimental data.

342   Then, since a lack of fit was detected in the fully constrained multigroup model, a series

343   of nested models were developed to detect which paths significantly improved the model

344   when released (Shipley, 2002). For this, we removed each path of the model one at a

345   time. The difference in the two maximum likelihood χ^2 statistics was used to test for a

346   difference in the value of a parameter between invasive and native species after

347   Bonferroni correction. The overall significance level of path coefficients and multigroup

348   analysis were carried out using AMOS 5.0 software (AMOS Development Corp., Mount

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349 Pleasant, SC, USA) whereas the rest of SEM analyses were performed with the SEPATH

350 procedure of the Statistica 7.0 (StatSoft, Inc) software package.

351

352 **Results**

353 *Fitness, mean trait values, phenotypic plasticity and phenotypic integration: invasive vs.*

354 *native*

355 Invasive species displayed higher biomass and survival than native species. These

356 differences were significant under medium nutrient, high nutrient and shade for biomass,

357 and shade for survival (Table 3). R/S ratio and PNUE significantly differed between

358 invasive and native species. Invasive species had lower R/S ratio and higher PNUE

359 values than native species across treatments (Table 3).

360 Invasive and native species showed similar R/S plasticity values across resource

361 levels. However, invasive species showed significantly higher PNUE plasticity than

362 native species from low to medium nutrient and from shade to sun but not from medium

363 to high nutrient (Table 3). Total biomass was not statistically significant when included

364 as a covariable, meaning that observed differences in R/S and PNUE plasticity were not a

365 consequence of an increase in plant size (R/S: low to medium nutrient $F_{1,39}$=2.04, p=0.53,

366 medium to high nutrient $F_{1,39}$=0.28, p=0.88, shade to sun $F_{1,39}$=5.31, p=0.20; PNUE: low

367 to medium nutrient $F_{1,39}$=2.77, p=0.46 , medium to high nutrient $F_{1,39}$=1.49, p=0.61,

368 shade to sun $F_{1,39}$=4.96, p=0.27). Finally, phenotypic integration was tended to be also

369 higher in invasive species, but this trend was only from medium to high nutrient (Table

370 3).

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372 *Structural equation modelling of phenotypic performance*

373 Goodness-of-fit tests for SEM indicated an overall good model fit in all invasive

374 and native models across treatments. χ^2 test was not significant at p>0.05, which implies

375 that the covariance structure specified by each model could not be rejected. Also, GFIs

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376   and NFIs index were higher or similar to 0.90 indicating that they provide an optimal fit

377   compared to a null model that assumes independence among all variables (Fig. 1B-G).

378   Importantly, we did not observe significant differences in the path coefficients described

379   below between invasive and native species when the three native species invasive

380   elsewhere were removed (i.e. probability of ΔML χ^2 between table 4 and table S3 did not

381   differ statistically).

382   From low to medium nutrient level, invasive and native species differed in the

383   way their increment of biomass was achieved. While PNUE plasticity had a significant

384   and positive direct effect in the increment of biomass for invasive species, PNUE mean

385   did so on the biomass increment of natives (Table 4). In turn, this increment of biomass

386   affected positively survival. Surprisingly, R/S and plasticity of R/S did not have a

387   significant effect on fitness in any group (Fig.1 B-C). Phenotypic integration of invasive

388   species was positively correlated with PNUE plasticity and negatively correlated with

389   R/S plasticity (Table 4). Interestingly, invasive species offset more than natives the direct

390   negative influence of PNUE plasticity on survival (path 4b, invasive = -0.46, native= -

391   0.26). They achieved so by the positive indirect influence of PNUE plasticity on biomass

392   (path 4a*path6=0.61*0.32=0.195) plus the positive direct influence of phenotypic

393   integration on survival (path5b=0.44), whereas natives had only a direct influence of

394   PNUE on survival through biomass (path2*path6=0.40*0.48=0.192) (Fig.1B-C).

395   From medium to high nutrient level, an opposite pattern regarding PNUE was

396   found in comparison to low to medium nutrient level. Now, the increment of biomass of

397   invasive species was positively driven by PNUE mean, and the increment of biomass of

398   native species was positively driven by PNUE plasticity. Further, R/S mean was

399   negatively related to an increment of biomass and the relative importance of this path did

400   not differ between invasive and native species (Table 4). Here, a negative effect of R/S is

401   indicates a smaller R/S (i.e higher S/R, higher above- than below-ground biomass) has a

402   positive effect on fitness. Moreover, the effect of phenotypic integration on the fitness of

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403   invaders highlights the complexity of observed relationships. On one hand, high

404   phenotypic integration values decreased survival, but on the other hand it diminished the

405   negative effect of R/S plasticity on survival, via the negative correlation between

406   integration and R/S plasticity (path c3) (Fig. 1D). This complexity reflected again the

407   ability of invasive species to offset the direct negative effect of plasticity and integration

408   on fitness. For native species in contrast, phenotypic integration directly increased both

409   survival and biomass and indirectly increased biomass through its positive correlation

410   with PNUE plasticity (Fig. 1E).

411   From shade to sun, invasive and native species did not significantly differ in their

412   path coefficients (i.e. goodness-of-fit did not improve significantly when a path

413   coefficient was released in multigroup comparison tests) (Table 4). Lower R/S

414   contributed to increased biomass and this in turn to increase survival. Increased biomass

415   was the direct consequence of high PNUE mean values, PNUE plasticity and phenotypic

416   integration. Also, phenotypic integration positively affected survival (Fig. 1F-G).

417   In summary, our results show that across resource gradients invasive and native

418   species achieve fitness in a similar way. Trait means had a higher influence on increased

419   biomass and survival than phenotypic plasticity and integration. In addition, increased

420   biomass had a consistent positive effect on survival. The physiological trait and its

421   plasticity (PNUE) had higher positive effect on fitness than the morphological (R/S) one.

422   Phenotypic integration, in turn, was positively correlated with PNUE plasticity but

423   negatively correlated with R/S plasticity. Regarding differences between invasive and

424   native species we found that first, there was a switch in the relative importance of PNUE

425   and PNUE plasticity for the increment of biomass across a nutrient gradient, and second,

426   invasive species offset more the few cases that plasticity and integration had a negative

427   effect on fitness.

428

429

Discussion

430 Invasiveness can be promoted either by higher values of certain traits, phenotypic
431 plasticity and/or phenotypic integration than natives, and by higher and positive relative
432 influence of these three aspects of plant functionality on fitness. Our results showed that
433 the relative importance of traits, plasticities and integration was similar between invasive
434 and native species, but they followed a hierarchy: traits means had a higher relative
435 importance for fitness than trait plasticity and plasticity had a similar importance to
436 integration. Thus, invasive species obtained higher biomass across resources gradients
437 and higher survival in the shade because of their general higher trait mean values, and
438 their higher PNUE plasticity. In addition, our multivariate framework also highlighted
439 that such differences in fitness were also attributable to a higher ratio of adaptive
440 responses. While traits mostly influenced a fitness gain across resources gradients,
441 phenotypic plasticity and phenotypic integration showed a mix of adaptive-non adaptive-
442 maladaptive responses.

443

444 *Multiple strategies promote fitness and reduce maladaptive responses.*

445 Invasive and native species altered traits, plasticity and integration in concert
446 rather than varying only one of these aspects of the functional strategy. Presumably, this
447 combination of strategies has been selected to augment the likelihood of achieving
448 fitness. However, it also entailed in a lesser extent maladaptive plastic and integrated
449 responses. These maladaptive responses may persist because of genetic correlations
450 among different components of the functional strategy under selection (Pigliucci *et al.*,
451 2006), where maladaptive plastic and integrated responses are compensated for by the
452 positive effects of other functional aspects.

453 Although less studied, maladaptive plastic responses can be common (van
454 Kleunen & Fischer, 2005; Valladares *et al.*, 2007). For instance, a negative influence of
455 plasticity on survival was found in four Iberian tree species grown along a light gradient

457 (Sánchez-Gómez *et al.*, 2006). However, still it is unclear how relevant are maladaptive

458 responses for species performance and how these responses translate to community

459 dynamics (Miner *et al.*, 2005). In our study maladaptive responses were important but

460 they were offset but the positive effect of other aspect of the functional strategy, where

461 invasive species did better. Instead of studying maladaptive responses in isolation, we

462 encourage the application of multivariate approaches such as the one followed here to test

463 how the variation in the ratio between adaptive and maladaptive responses affect overall

464 plant fitness and hence their invasive potential.

465

466 *Little support for different functional strategies between invasive and native species*

467 *along resource gradients*

468 Invasive and native species differed in the relative importance of PNUE and PNUE

469 plasticity along the nutrient gradient. Only invasive species support the hypothesis that

470 higher plasticity of traits associated with resource use efficiency (i.e. PNUE plasticity)

471 are crucial for achieving higher biomass from limiting to non-limiting resource levels (i.e.

472 low to medium nutrient), as Funk (2008) found. However, under high resource

473 availability (i.e. medium to high nutrient), high PNUE was more important for achieving

474 high biomass. We did not find support to the hypothesis of the higher relative importance

475 of PNUE plasticity versus PNUE mean values for invasive species from shade to sun, in

476 contrast to findings of other studies (Poorter, 1999; Sánchez-Gómez *et al.*, 2006). These

477 discrepancies may be due to the relatively high light of our shade treatment (20% of full

478 sunlight) as compared to those used in these studies (6-10%).

479 Surprisingly, morphological plasticity, represented by R/S plasticity, did not

480 influence fitness. This contrasts with other studies which reported that plasticity of

481 morphological traits, such as R/S, SLA, or LAR, is usually involved in adaptive

482 responses to light or nutrient shifts (Valladares *et al.*, 2000; Dudley, 2004; Hodge, 2004;

483 Funk, 2008). Perhaps, our results are due to differences time scale as morphological

484   plasticity has a slower response-time than physiological plasticity (Funk *et al.*, 2007).

485   Given that we measured fitness after a short time (6 months), it might be more influenced

486   by the more dynamic physiological plasticity. Yet, it is not clear the implications for plant

487   performance of displaying a more or less dynamic plasticity (Funk *et al.*, 2007), but

488   presumably the future costs associated with a more dynamic plasticity (such as PNUE

489   plasticity) are less than the costs associated with a less dynamic plasticity (such as R/S

490   plasticity). For instance, high R/S plasticity to shade can generate a maladapted

491   phenotype to a future drought (Valladares *et al*., 2007). Another explanation for these

492   results might be that our resource gradients were not wide enough to elicit a significant

493   R/S variation in six months. However, this latter explanation seems less likely because

494   analogous studies with similar growth lengths and resource gradients have obtained

495   significant results (Sánchez-Gómez *et al.*, 2006; Funk, 2008).

496

497   *The importance of phenotypic integration for invasiveness: new evidence*

498   Phenotypic integration also followed a pattern of adaptive, non-adaptive and

499   maladaptive mix. Thus, their relative importance for invasiveness can be considered

500   similar to plasticity but lower than trait means. Interestingly, phenotypic integration

501   primarily promoted survival, which may be important for establishment success of

502   invasive taxa. However, our results suggest that the most important role for invasiveness

503   was the consistent positive effect on the expression of adaptive PNUE plasticity. In this

504   sense, we support, but only partially, the notion that phenotypic integration may constrain

505   phenotypic plasticity (see suggestions by Gianoli, 2004; Gianoli & Palacio-López, 2009).

506   When significant, morphological plasticity (R/S ratio) was negatively correlated with

507   phenotypic integration. In contrast, physiological plasticity (PNUE) was not. Our

508   discrepancy with Gianoli & Palacio-López, (2009) may be due to the fact that they only

509   correlated phenotypic integration to morphological plasticity. We hypothesize that a more

510   integrated phenotype responds to environmental variation with traits of faster plastic

511   responses because highly dynamic plasticity does not typically entails changes in other

512   traits at higher levels of organization. In contrast, a plastic response in a morphological

513   trait such as R/S plasticity, which determines key aspects of plant architecture, may entail

514   a plastic response in other traits at lower levels of organization in a cascade effect.

515   This link between integration and highly dynamic plasticity may be important for

516   invasiveness because exotic plants render faster adaptive plastic responses with lower risk

517   of mortality.

518

519   *Conclusion*

520   Previous works have shown the importance of particular traits and trait plasticity as

521   determinants of invasiveness. But their relative importance was not explored because

522   they were not explicitly studied in combination. Besides, the role of phenotypic

523   integration on invasiveness remained unexplored. According to our multivariate models,

524   we conclude that fitness in both invasive and native species is jointly determined by trait

525   mean values, their phenotypic plasticity and phenotypic integration, following a fitness

526   hierarchy. Invasive species had higher fitness than natives because i) they showed higher

527   PNUE and lower R/S, which was positively associated with fitness along gradients, ii)

528   they showed higher physiological plasticity, which was also associated with higher

529   biomass, and iii) they offset more the cases when plasticity and integration had a negative

530   influence on fitness. Although the relative contribution of these three components of the

531   functional strategy to invasiveness may be modulated by other factors associated with

532   human activities (Pyšek *et al.*, 2009), multivariate approaches such as the one followed

533   here are very promising to disentangled which factors are promoting plant fitness and

534   hence the invasion potential of exotic species.

535

536   **Acknowledgements**

545

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708

Supporting information

710   Additional supporting information may be found in the online version of this article

711   **Appendix S1** UTM coordinates where seeds were obtained

712   **Table S1** Mean and standard errors for each trait of each species in each environment.

713   **Appendix S2** Detailed description of the 31 traits measured

714   **Table S2** Trait loadings extracted by a Principal Component Analysis (PCA) in order to

715   calculate phenotypic integration.

716   **Figure S1** Allometric relationship between root and shoot (R/S) and between maximum

717   photosynthetic rate and leaf nitrogen content per unit of area (PNUE) for invasive and

718   native species along resource gradients.

719   **Table S3** Multigroup comparison of path coefficients among invasive and native species

720   and between resources treatments after excluding the three native species invasive

721   elsewhere.

722

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Figure legends

Figure 1 A) Aprioristic structural equation model of the causal relationship between plant traits, traits plasticity, phenotypic integration and fitness. U_1 and U_2 represent the unexplained variance of dependent variables. Straight lines represent simple regression between variables whereas curve lines denote correlation. Solid lines indicate positive effect whereas dashed lines negative. Line thickness indicates relative path importance. For illustrative purposes, non significant path coefficients are colored in grey. Left row correspond with invasive species models (A, D, F), while right row to natives models (C, G, E). Path-ways of simple regression are named from 1 to 6 and those of correlations from c1 to c4. Model fitting and path coefficients are shown from B) to G). An asterisk denotes significant path coefficient at $p<0.05$. Significant differences between invasive and native path coefficients value across environments are described in Table 4.

Tables

Table 1 Invasive-native phylogenetically and ecologically related pairs selected for the experiment. The taxonomic family, growth form and habitat are indicated in columns for each species pair. Also native species invasive elsewhere are denoted by an asterisk

Pair nº	Family	Invasive species	Native species	Growth Form	Habitat
1	Fabaceae	*Acacia melanoxylon*	*Anagyris foetida*	Woody	Shrubland
2	Malvaceae	*Abutilon theophrasti*	*Althaea officinalis*	Herbaceous	Grassland
3	Onagraceae	*Oenothera biennis*	*Epilobium hirsutum*	Herbaceous	Grassland
4	Fabaceae	*Gleditsia triacanthos*	*Colutea arborescens*	Woody	Woodland
5	Solanaceae	*Nicotiana glauca*	*Lycium intricatum*	Woody	Shrubland
6	Fabaceae	*Sophora japonica*	*Ceratonia siliqua*	Woody	Woodland
7	Ulmaceae	*Ulmus pumila*	*Ulmus minor*	Woody	Woodland
8	Anacardiaceae	*Schinus molle*	*Pistacia terebinthus*	Woody	Woodland
9	Elaeagnaceae/ Rhamnaceae	*Elaeagnus angustifolia*	*Rhamnus alaternus*	Woody	Shrubland
10	Asteraceae	*Baccharis halimifolia*	*Dittrichia viscosa**	Woody	Shrubland
11	Pinaceae	*Pinus radiata*	*Pinus pinaster**	Woody	Woodland
12	Solanaceae	*Datura stramonium*	*Hyoscyamus niger*	Herbaceous	Grassland
13	Tropaeolaceae/ Brassicaceae	*Tropaeolum majus*	*Capparis spinosa*	Herbaceous	Shrubland
14	Solanaceae	*Solanum bonariense*	*Solanum nigrum*	Herbaceous	Grassland
15	Simaroubaceae/ Rutaceae	*Ailanthus altissima*	*Cneorum tricoccon*	Woody	Shrubland
16	Myrtaceae	*Eucalyptus globulus*	*Myrtus communis*	Woody	Woodland
17	Poaceae	*Cortaderia selloana*	*Phragmites communis*	Herbaceous	Grassland
18	Asteraceae	*Achillea filipendulina*	*Achillea millefolium**	Herbaceous	Grassland
19	Poaceae	*Ampelodesmos mauritanica*	*Stipa tenacissima*	Herbaceous	Grassland
20	Oxalidaceae	*Oxalis pes-caprae*	*Oxalis corniculata*	Herbaceous	Grassland

Table 2 Variables and descriptions of the traits measured. Effective quantum yield, quenchings and electronic transportation rate were measured at non-saturating light level (150 µmol photon m^{-2} s^{-1}) and saturating light level (1900 µmol photon m^{-2} s^{-1}). Details of trait measurements are included in the Appendix S2.

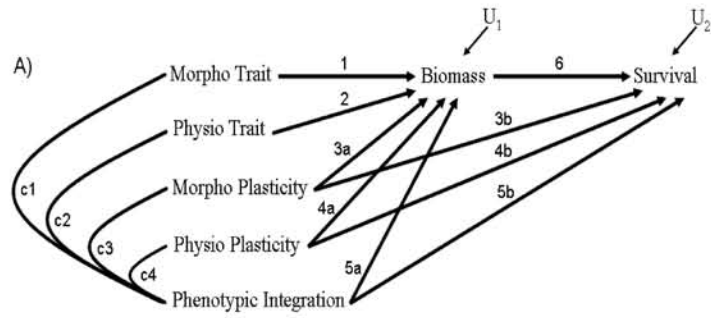| Variable	Description	Units
Canopy structure		
H	Height	cm
CA	Crown area	cm^2
SD	Stem diameter	mm
NL	Number of leaves	-
Allometry		
LWR	Leaf weight ratio	g leaf g^{-1} plant
SWR	Stem weight ratio	g stem g^{-1} plant
RWR	Root weight ratio	g root g^{-1} plant
LAR	Leaf area ratio	cm^2 leaf g^{-1} plant
R/S	Root/Shoot ratio	g root g^{-1} stem and leaf
SLA	Specific leaf area	cm^2 leaf g^{-1} leaf
Leaf physiology		
A_{max}	Maximum photosynthetic rate at saturation light	µmol CO2 m^{-2} s^{-1}
iWUE	Instantaneous water use efficiency	µmol CO2 mol^{-1} H$_2$O
PNUE	Photosynthetic nitrogen use efficiency	µmol CO2 mol^{-1} N s^{-1}
N_{area}	Leaf nitrogen content per area	mg N cm^{-2} leaf
N_{mass}	Leaf nitrogen concentration	mg N g^{-1} leaf
R_{dark}	Plant respiration	µmol CO2 m^{-2} s^{-1}
Quantum yield (Φ)	Apparent maximum quantum yield	µmol CO2 µmol^{-1} photon
Curvature factor (Θ)	Light curve convexity	-
Compensation point (Γ)	Light compensation point	µmol photon m^{-2} s^{-1}
Saturation point (Ic)	Light saturation point	µmol photon m^{-2} s^{-1}
F_v/F_m	Ratio of variable to maximum fluorescence	
Φ_{PSII} (at 150, 1900)	Effective quantum yield of PSII	-
qP (at 150, 1900)	Photochemical quenching	-
qN (at 150, 1900)	Quenching non-photochemical associated to radiant energy dissipation	-
NPQ (at 150, 1900)	Quenching non-photochemical associated to non-radiant energy dissipation	-
ETR (at 150, 1900)	Electronic transport rate	µmol e$^-$ m^{-2} s^{-1}
Fitness related variables		
Survival	Percentage of survival during growth	%
Total biomass	Total above and below ground biomass	g plant

Table 3 Statistical differences in Root/Shoot ratio (R/S), photosynthetic nitrogen use efficiency (PNUE), R/S plasticity, PNUE plasticity, phenotypic integration and fitness estimators (biomass, and survival) between invasive and native species. Second row shows whether traits were calculated within or between two treatments. F and p-values correspond to PERMANOVA analyses. d.f=39. Mean ± standard error are also shown. * $p<0.05$, **$p<0.01$, ***$p<0.001$, ns non significant.

Functional strategy	Resource level/ Change in resource level	Invasive	Native	F, p
R/S	Low	1.41±0.14	**1.92±0.17**	10.07*
	Medium/Sun	1.69±0.12	**2.51±0.16**	15.72**
	High	1.34±0.17	**1.94±0.21**	12.35**
	Shade	0.52±0.09	**0.68±0.09**	8.43*
PNUE (μmol CO_2 mol^{-1} N s^{-1})	Low	**134.06±10.46**	115.40±10.01	8.62*
	Medium/Sun	**168.67±9.31**	135.24±6.60	14.17**
	High	**191.02±11.15**	149.29±11.24	17.77**
	Shade	**172.39±8.88**	145.83±9.92	14.43**
R/S plasticity	Low to Medium	0.07±0.04	0.08±0.06	2.33 ns
	Medium to High	0.13±0.07	0.18±0.06	3.26 ns
	Shade to Sun	0.46±0.09	0.52±0.05	1.19 ns
PNUE plasticity	Low to Medium	**0.37±0.09**	0.10±0.05	18.81***
	Medium to High	0.15±0.04	0.18±0.05	4.96 ns
	Shade to Sun	**0.49±0.05**	0.21±0.08	22.26***
Phenotypic Integration	Low to Medium	0.30±0.09	0.27±0.10	0.25 ns
	Medium to High	**0.33±0.07**	0.20±0.05	13.48**
	Shade to Sun	0.26±0.03	0.22.±0.06	3.15ns
Biomass (g)	Low	0.646±0.077	0.543±0.121	2.13 ns
	Medium/Sun	**2.619±0.384**	1.904±0.375	13.17**
	High	**6.441±0.724**	4.215±0.653	20.32***
	Shade	**1.360±0.270**	0.831±0.176	8.94*
Survival (%)	Low	0.89±0.03	0.88±0.03	0.45 ns
	Medium/Sun	0.95±0.02	0.90±0.02	6.74ns
	High	0.93±0.02	0.88±0.03	3.18ns
	Shade	**0.93±0.01**	0.82±0.04	14.49**

Table 4 Multigroup comparison of path coefficients among invasive and native species and between resources treatments. The first row shows the maximum likelihood χ^2 estimates (ML χ^2) from constraining all free parameters to the same value. The following rows are the effect on χ^2 of releasing each single free parameter one at a time. The difference between the constrained model and the rest are given as ΔML χ^2, the p-value indicates the probability that the release of that parameter improves the model significantly. A significant value p-value indicates that the relative path contribution to the model is different between invasive and native species. See Fig. 1A for path codes. Bonferroni-corrected p-value threshold 0.05/15=0.003.

Free parameters for which between-group equality constraint was released	Low to Medium			Medium to High			Shade to Sun		
	ML χ^2	ΔML χ^2	Probability of ΔML χ^2	ML χ^2	ΔML χ^2	Probability of ΔML χ^2	ML χ^2	ΔML χ^2	Probability of ΔML χ^2
None	204.841			210.501			90.034		
Path 1 (R/S to Biomass)	204.725	0.115	0.672	210.485	0.016	0.793	88.596	1.438	0.232
Path 2 (PNUE to Biomass)	192.514	12.327	**0.001**	200.474	10.027	**0.001**	86.096	3.937	0.042
Path 3a (R/S *RDPI* to Biomass)	200.561	4.280	0.043	210.116	0.385	0.404	88.550	1.483	0.224
Path 3b (R/S *RDPI* to Survival)	202.698	2.142	0.146	206.483	4.018	0.029	88.464	1.569	0.218
Path 4a (PNUE *RDPI* to Biomass)	193.228	11.613	**0.001**	198.887	11.614	**0.001**	88.642	1.392	0.241
Path 4b (PNUE *RDPI* to Survival)	204.130	0.712	0.379	210.402	0.099	0.602	88.828	1.205	0.277
Path 5a (Phenotypic Integration to Biomass)	203.491	1.350	0.241	208.247	2.254	0.119	88.596	1.438	0.232
Path 5b (Phenotypic Integration to Survival)	187.533	17.308	**0.001**	186.972	23.529	**0.001**	89.963	0.071	0.858
Path 6 (Biomass to Survival)	202.195	2.647	0.108	203.900	6.601	0.007	89.869	0.164	0.739
Path C1 (R/S and Phenotypic Integration)	200.444	4.398	0.041	204.880	5.622	0.013	88.279	1.755	0.184
Path C2 (PNUE and Phenotypic Integration)	187.903	15.841	**0.001**	196.623	14.897	**0.001**	83.726	6.307	0.010
Path C3 (R/S *RPDI* and Phenotypic Integration)	191.345	13.496	**0.001**	190.545	19.956	**0.001**	88.817	1.217	0.275
Path C4 (PNUE *RDPI* and Phenotypic Integration)	200.495	4.345	0.059	204.375	6.126	0.010	85.136	4.898	0.023
Error variance of increment biomass	122.787	82.055	**0.001**	206.471	4.030	**0.001**	85.565	4.469	0.030

A)

U₁   U₂

Morpho Trait —1→ Biomass —6→ Survival
Physio Trait
Morpho Plasticity
Physio Plasticity
Phenotypic Integration

Invasive Species   Native Species

Low to Medium Nutrient

B)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:14.10
CFI: 0.92
NFI: 0.90

C)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:14.17
CFI: 0.91
NFI: 0.90

Medium to High Nutrient

D)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:13.85
CFI: 0.93
NFI: 0.90

E)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:14.08
CFI: 0.92
NFI: 0.90

Shade to Sun

F)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:9.13
CFI: 0.96
NFI: 0.95

G)
R/S
PNUE
R/S Plasticity
PNUE Plasticity
Phenotypic Integration
Increment Biomass → Survival
χ²:9.83
CFI: 0.94
NFI: 0.92