

The relative importance for plant invasiveness of trait means, and their plasticity and integration in a multivariate framework

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Summary

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- Functional traits, their plasticity and their integration in a phenotype have profound impacts on plant performance. We developed structural equation models (SEMs) to evaluate their relative contribution to promote invasiveness in plants along resource gradients.
- We compared 20 invasive–native phylogenetically and ecologically related pairs. SEMs included one morphological (root-to-shoot ratio (R/S)) and one physiological (photosynthesis nitrogen-use efficiency (PNUE)) trait, their plasticities in response to nutrient and light variation, and phenotypic integration among 31 traits. Additionally, these components were related to two fitness estimators, biomass and survival.
- The relative contributions of traits, plasticity and integration were similar in invasive and native species. Trait means were more important than plasticity and integration for fitness. Invasive species showed higher fitness than natives because: they had lower R/S and higher PNUE values across gradients; their higher PNUE plasticity positively influenced biomass and thus survival; and they offset more the cases where plasticity and integration had a negative direct effect on fitness.
- Our results suggest that invasiveness is promoted by higher values in the fitness hierarchy – trait means are more important than trait plasticity, and plasticity is similar to integration – rather than by a specific combination of the three components of the functional strategy.

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 noninvasive/native species. For instance, high maximum photosynthetic rate, high specific leaf area (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 because 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 addition, phenotypic integration may increase survival by reducing the cost of maladaptive and/or nonadaptive plastic traits (van Kleunen & Fischer, 2005; Poot & Lambers, 2008). However, our empirical

knowledge about phenotypic integration is scarce and more work is needed for a better understanding of the role of phenotypic integration in plant fitness (Pigliucci & Preston, 2004). For instance, previous empirical studies found a negative relationship between phenotypic plasticity and phenotypic integration (Gianoli, 2004; Gianoli & Palacio-López, 2009), a surprising finding because, theoretically, both can favor plant fitness.

Rather than continuing to study separately whether particular traits, their plasticity or their integration are linked to invasiveness, it is more relevant to have a solid knowledge about how these three aspects of the plant strategy promote plant fitness. To promote a rapid exclusion, exotic species must show high fitness differences with native species (MacDougall *et al.*, 2009). This may be achieved by adequately responding to fluctuations in a given resource, but also by avoiding potential future costs resulting from nonadaptive responses (van Kleunen & Fischer, 2005; Valladares *et al.*, 2007). For instance, high fitness differences as a result of adaptive plastic responses may be more likely to occur in a less constrained phenotype (i.e. with low phenotypic integration), or in a highly stressful environment (Richards *et al.*, 2006). Moreover, superior performance may be underpinned by a combination of higher mean trait and greater adaptive plasticity (Godoy *et al.*, 2011).

In a previous study comparing 20 invasive–native phylogenetically and ecologically related plant pairs, we observed that invasive species showed higher biomass gain and survival after 6 months of growth than native species (Godoy *et al.*, 2011). Here, our aims are to unravel how trait means, phenotypic plasticity and phenotypic integration contribute to fitness differences between invasive and native species; and how the relative importance of these three components of the functional strategy varies along resource gradients. These aims were tackled in a multivariate way by means of structural equation modeling (SEM) (see Shipley, 1999, 2002, 2004 for details).

Our aprioristic model posits that both key morphological and physiological traits, and their corresponding plasticities, directly affect fitness (as found by Daehler, 2003; Funk, 2008). We assume that biomass influences the survival rate, and both are critical surrogates of fitness for perennial plants or in short-term studies (Sultan, 2001). We expect a negative correlation between phenotypic integration and morphological and physiological plasticities (Gianoli, 2004; Gianoli & Palacio-López, 2009). Thus, phenotypic integration may increase biomass and survival as opposed to trait plasticity (see Fig. 1a for SEM structure). We hypothesize that invasive species will show higher fitness because the overall contribution of their trait means and trait plasticity is higher. We also hypothesize that higher adaptive trait plasticity will contribute more to the invasive species' fitness under resource-limited conditions, while particular trait values will be more relevant for invasive species to achieve fitness in nonlimiting parts of resource gradients (e.g. Funk, 2008). Finally, we may also expect that phenotypic integration have a positive effect on the increment of biomass and survival, from limiting to nonlimiting conditions (Schlichting, 1989; Waitt & Levin, 1993; Gianoli, 2004). However, we do not have any previous support to hypothesize that this positive effect will be higher in invasive species.

Materials and Methods

Species selection and experimental set-up

We selected 20 clearly invasive exotic species in the Iberian Peninsula (*sensu* Pyšek *et al.*, 2004), as they are local dominants in some native ecosystems (Valéry *et al.*, 2008), and have a potential impact on the native ecosystems (transformer species, *sensu* Richardson *et al.*, 2000) (Table 1). They represent a broad range of taxonomic groups, habitat preferences (woodlands, shrublands, grasslands and riparian areas) and growth forms (annual and biannual herbs, shrubs and trees) (Sanz-Elorza *et al.*, 2004). Then, we paired each invasive species with one closely related native species of the Iberian Peninsula based on a suite of phylogenetic and ecological criteria: the native species had to belong to the same family as the invasive species, which was achieved in 17 of the 20 pairs; they had to have the same growth form (achieved in all pairs except number 15 and 16, in which invasive species were trees and natives shrubs); they had to coexist in the same habitat type in the Iberian Peninsula and the same successional community stage; and they had to be recorded as co-occurring at least once in Spain (Table 1). We consulted the extensive herbarium database at Universidad Complutense de Madrid (MACB, founded 1968) to check for co-occurrence within pairs. Native species with small distribution ranges, that are rare or have an endangered status were excluded. Moreover, only three of the 20 native species selected were recorded as invasive elsewhere. Hence the native species set can be considered mostly noninvasive as well.

We designed a nonfactorial experiment with two different resource gradients: a nutrient gradient with three supply levels (low/medium/high) and a light gradient with two intensities (shade/sun). In the nutrient gradient, 'low' was equivalent to 0.010 g N per plant, 'medium' to 0.085 g N per plant and 'high' to 0.245 g N per plant. In the light gradient, 'shade' was equivalent to 20% full radiation (max. photosynthetic photon flux density (PPFD), 350–500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with light quality modified to red/far red = 0.8 (to mimic natural shade effects on the light spectrum by establishing layers of green cloth), and 'sun' was equivalent to 50% full radiation (max. PPFD, 950–1050 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with no red/far red modification. We avoided a 100% light intensity for the 'sun' setting because the high irradiance of the experimental site during the summer could compromise the viability of the experiment. In the nutrient gradient, light was kept constant at 50% of full radiation (950–1050 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and in the light gradient, N doses were kept constant at medium N concentration (0.085 g N per plant). This combination of factors represents *a priori* a change in the resource availability from limiting to nonlimiting (low to medium nutrient supply, and shade to sun intensity) to two levels of nonlimiting resources (medium to high nutrient supply).

Each species per resource level was replicated three times (blocks) to control for possible microenvironmental variations and each block contained 12 individuals per species. In total, we used a total of 5760 plants, 144 per species (40 species \times four treatments \times three blocks \times 12 plants each block). Plants were

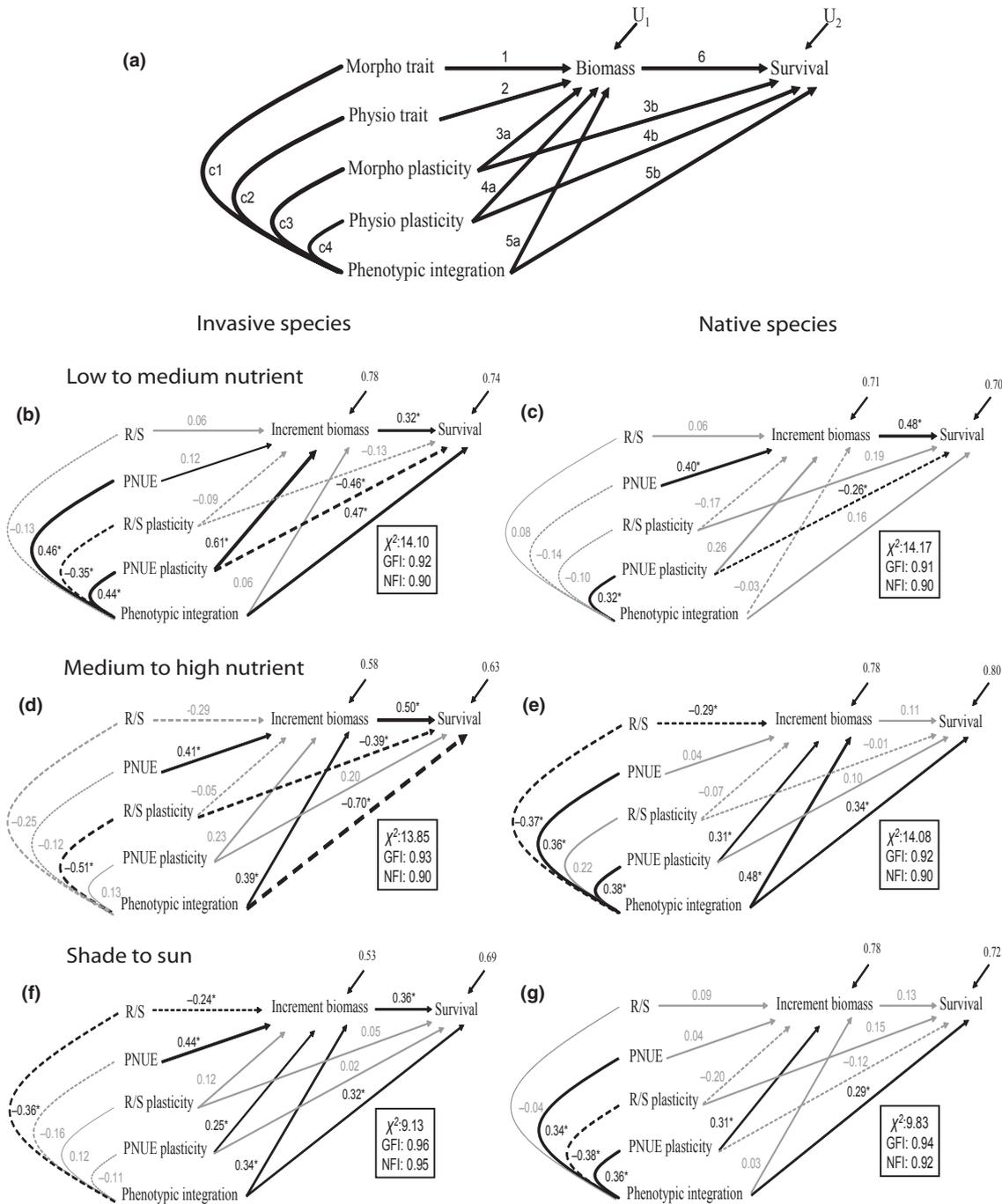


Fig. 1 (a) Aprioristic structural equation model of the causal relationship among plant traits, trait plasticity, phenotypic integration and fitness. U_1 and U_2 represent the unexplained variance of dependent variables. Straight lines represent simple regression between variables whereas curve lines denote correlation. Solid lines indicate positive effect whereas dashed lines indicate negative effect. Line thickness indicates relative path importance. For illustrative purposes, nonsignificant path coefficients are coloured in gray. Morpho, morphological; physio, physiological. Panels (b), (d) and (f) correspond to invasive species models while Panels (c), (e) and (g) correspond to native models. Pathways of simple regression are numbered 1 to 6 and those of correlations are numbered c1 to c4. Model-fitting and path coefficients are shown from (b) to (g). An asterisk denotes a significant path coefficient at $P < 0.05$. Significant differences between invasive and native path coefficient values across environments are described in Table 4. GFI, goodness-of-fit index; NFI, Bentler–Bonett normed-fit index; PNUE, photosynthesis nitrogen-use efficiency; R/S, root-to-shoot ratio.

grown from seeds in 11 pots (QP 12T/18; Projar, Valencia, Spain) from February to September in each of the two years (2005, 2006) during which the study was carried out. Seeds were obtained from commercial suppliers or through field collection. In both cases, seeds came from locations where the exotic species

are clearly invasive. In the case of commercial suppliers, seeds were certified to come from one single location. When collected in the field, seeds were gathered from 15–20 haphazardly chosen plants within one population. Population delimitation followed a similar procedure to other studies, such as Schlaepfer *et al.*

Table 1 Invasive–native phylogenetically and ecologically related pairs selected for the experiment

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

*Native species invasive elsewhere.

The taxonomic family, growth form, and habitat are indicated in columns for each species pair.

(2010) (i.e. one population was defined as continuous stands of species covering an area between 4 and 40 000 m² and separated from other populations by at least 10 km; see Supporting Information, Notes S1 for locations). Just after seed germination, we fertilized seedlings with a Plantacote mix 6 month slow-release fertilizer 14–90–15 N–P–K, (Aglukon Spezialdünger GMBH & Co. KG, Dusseldorf, Germany). We used a slow-release fertilizer to ensure that plants had available N throughout the experiment. The main N compound was ammonium nitrate (NH₄NO₃) (85%). Pure vermiculite was used as substrate to ensure that the fertilizer was the only source of nutrient supply. The gravimetric soil water content in the pots was maintained at > 30%. Local air temperature and available PPFD were recorded every 5 min throughout the growing season with a data logger (HOBO model H08-006-04; Onset, Pocasset, MA, USA) and self-made external sensors that were cross-calibrated with a Li-Cor 190SA sensor (Li-Cor, Lincoln, NE, USA). The mean daily temperature was 17.3°C (ranging from 9.6 to 22.5°C) and the mean daily PPFD (400–700 nm) over the summer was 41 mol m² d⁻¹, which is equivalent to full sunlight.

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 (Tables 2, 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 in the SEM analyses (see the section ‘Structural equation modeling of phenotypic performance’); and secondly, 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 leaf area ratio (LAR) and root weight ratio (RWR) are associated with light and nutrient acquisition, respectively), plant competition (e.g. high rate of maximum photosynthesis and F_v/F_m are associated with fast growth and optimal physiological state), and stress tolerance (e.g. high PNUE is associated with high plant performance in N-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 as a result of 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 the phenotypic plasticity of each trait and species using the relative distance plasticity index (RDPI) (Valladares *et al.*, 2006). Before any analysis, trait data were log-transformed (log[x]) to avoid differences in scale within and between traits. We used RDPI because it is highly correlated with other indices of phenotypic plasticity commonly used in the literature (Valladares *et al.*, 2006); and also because it has the advantage of being the only index that provides a statistical distribution of relative distances that can be implemented into SEM analyses (see the section ‘Structural equation modeling of phenotypic performance’) and phenotypic integration estimations. The number of relative distances was equal to the number of replicates per treatment to avoid pseudoreplication. Each relative distance was calculated as the absolute value of the trait distance between two randomly selected individuals of the same species belonging to two different environments, divided by the sum of their trait values.

Table 2 Variables and descriptions of the traits measured

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-to-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 CO ₂ m ⁻² s ⁻¹
iWUE	Instantaneous water-use efficiency	μmol CO ₂ mol ⁻¹ H ₂ O
PNUE	Photosynthetic nitrogen-use efficiency	μmol CO ₂ mol ⁻¹ N s ⁻¹
N _{area}	Leaf N content per area	mg N cm ⁻² leaf
N _{mass}	Leaf N concentration	mg N g ⁻¹ leaf
R _{dark}	Plant respiration	μmol CO ₂ m ⁻² s ⁻¹
Quantum yield (ϕ)	Apparent maximum quantum yield	μmol CO ₂ μmol ⁻¹ photon
Curvature factor (Θ)	Light curve convexity	–
Compensation point (Γ)	Light compensation point	μmol photon m ⁻² s ⁻¹
Saturation point (I _c)	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)	Nonphotochemical quenching associated with radiant energy dissipation	–
NPQ (at 150, 1900)	Nonphotochemical quenching associated with nonradiant 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

Effective quantum yield, quenchings and electronic transportation rate were measured at nonsaturating light intensity (150 μmol photon m⁻² s⁻¹) and saturating light intensity (1900 μmol photon m⁻² s⁻¹). Details of trait measurements are included in Supporting Information, Notes S2.

Additionally, we obtained an overall standardized RDPI value, one per species and trait, ranging from 0 (no plasticity) to 1 (maximum plasticity) by summing all relative distances obtained and dividing by the total number of distances (Valladares *et al.*, 2006). These standardized RDPI values were used to test for differences in trait plasticity between species (see the following section). For the nutrient treatment, we calculated the RDPI

from low to medium nutrient supply and medium to high nutrient supply. For the light treatment, the RDPI was calculated from shade to sun.

We defined phenotypic integration as the joint variation of two different traits in response to an environmental change. We estimated phenotypic integration at the level of species as the percentage of significant correlated plastic responses among traits between two treatments (i.e. low to medium nutrient supply, medium to high nutrient supply, shade to sun) (Schlichting, 1989; Schlichting & Pigliucci, 1998). Thus, phenotypic integration varies from 0 (no integration) to 1 (full integration). The number of plasticity replicates (i.e. number of relative distances obtained with RDPI), for estimating whether plastic responses among two traits were correlated, varied from three to nine for each species and treatment depending on the trait measured (Notes S2).

To our knowledge, this is the first study that estimates phenotypic integration in such a high number of traits and this may impose a methodological tradeoff. On one hand, this has the advantage of increasing the likelihood that traits accounting for phenotypic integration will be included. On the other hand, this has the disadvantage of inflating the total number of potential correlations, and hence we might underestimate the value of phenotypic integration. To handle this methodological tradeoff, we constrained the set of correlations to major axes of variation with functional meaning and we then averaged the percentage of correlated plastic responses for these axes to obtain an overall estimation of phenotypic integration per species.

We obtained three major axes of functional variation by performing a principal component analysis (PCA) (see PCA loadings, Table S2). The first axis (PC1) can be interpreted as a measure of physiological processes occurring on the leaves at molecular and electronic levels. It was positively related to photosynthetic machinery traits (e.g. maximum photosynthetic rate (A_{max}) and photochemical quenching (qP) reflect chlorophyll pigments) and negatively related to traits reflecting high contents of photoprotective pigments (e.g. nonphotochemical quenching associated with radiant energy dissipation (qN) and nonphotochemical quenching associated with nonradiant energy dissipation (NPQ) reflect xanthophylls pigments). The second axis (PC2) grouped the roles that leaf area and leaf N content play at different plant scales. This axis described important functional tradeoffs such as the negative correlation between SLA and leaf N content per area (N_{area}) found for the leaf economics spectrum (Wright *et al.*, 2004) and the negative correlation between water-use efficiency (iWUE) and leaf area allometry (SLA, LAR) (Reich *et al.*, 1989; Poorter *et al.*, 1990). The third axis (PC3) accounted for the contribution that roots make to the total plant biomass and described the tradeoff between below- and above-ground growth (Weiner, 2004).

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

We performed PERMANOVA analyses to test for differences between invasive and natives in: fitness estimators (biomass and

survival); R/S and PNUE mean values; R/S and PNUE plasticity; and phenotypic integration. We selected the PERMANOVA approach because it permits pairwise comparison at different phylogenetic levels, in agreement with our experimental design, and also because we could not always reach the assumptions of normality and homocedasticity of the data and its residuals (Anderson, 2001, 2005). We performed an analysis for each variable considering invasive/native status and nutrient/light treatments as fixed-factor, block as a random-factor and phylogenetic distance within pairs as a covariable. Analyses were conducted within treatments (R/S and PNUE) and between treatments (R/S plasticity, PNUE plasticity and phenotypic integration). Additionally, total biomass was also included as a covariate when analyzing differences in R/S and PNUE plasticity to check whether plastic responses were a mechanistic consequence of an increase in plant size (i.e. apparent plasticity) (Dudley, 2004; Funk, 2008). In all cases, differences between both groups and *post hoc* comparisons were estimated using the Bray–Curtis dissimilarity distance from 9999 permutations. The phylogenetic distance from one species to another for each of the species pairs was calculated through to the first common ancestor to both species using the plant phylogenetic supertree described by Soltis *et al.* (2000) and modifications by Bremer *et al.* (2003).

Structural equation modeling of phenotypic performance

Structural equation modeling provides an aprioristic-statistical approach that can be used to unravel the linking structure of traits that are correlated in a multivariate way based on previous knowledge (Shipley, 2004). We used SEM to investigate the relative contribution of mean trait values, phenotypic plasticity, and phenotypic integration to fitness differences between invasive and native species along resource variation; and also to disentangle direct from indirect effects of the three properties on fitness. The overall causal structure relating these components of the functional strategy was introduced earlier (Fig. 1a).

We selected R/S and PNUE as the key morphological and the physiological traits to predict plant fitness because of their importance in competition and stress tolerance and because they showed allometric plastic responses to resource variation (Weiner, 2004) (Fig. S1). The R/S ratio was highly correlated with LAR ($r > 0.80$, $df = 479$) and R/S and LAR plasticities were also correlated ($P < 0.01$) (from low to medium nutrient supply (L–M), $r = 0.76$ from medium to high nutrient supply (M–H), $r = 0.67$ from shade to sunlight (SH–S), $r = 0.82$, $df = 119$). The capacity to capture soil resources is dependent on R/S (Hodge, 2004) and the ability to capture light for photosynthesis is related to their LAR (Valladares *et al.*, 2002). High plasticity of R/S and LAR are often considered as strategies to maximize the capture of limited resources, such as light, nutrients or water (Valladares *et al.*, 2002; Poot & Lambers, 2008). PNUE integrates N leaf concentration (N_{mass}) and maximum photosynthetic rate (A_{max}). Thus, it was highly correlated with both physiological traits and their plasticities were also correlated. PNUE is also correlated with SLA (Poorter & Evans, 1998), as was found in our experiment ($r = 0.73$, $P < 0.05$, $df = 479$). PNUE provides

an insight into the efficiency of photosynthetic machinery (Poorter & Evans, 1998) and its plasticity is highly related to the competitive ability of plants (Funk, 2008).

To address the fact that we needed to analyze trait values (obtained within a level of resources) with plasticity and integration values (obtained between two levels), we fitted the model with the trait values of the level with higher resources. For instance, we used R/S and PNUE values from the medium nutrient treatment when the SEM model was performed from low to medium nutrient supply, and the same procedure was done from medium to high nutrient supply and from shade to sun. Biomass in the high resource level and differences in biomass between resource levels were highly correlated across treatments ($r > 0.85$, $P < 0.001$, $df = 359$); hence, the increase in biomass between treatments was included in the SEM models (Fig. 1a).

We assessed whether our aprioristic SEM fitted the data by a series of goodness-of-fit tests, which compared the observed covariance matrix with that derived from the model (Shipley, 2002). First, we performed a χ^2 test to evaluate the goodness-of-fit of our model. However, given that our data were not always adjusted to a multinormal distribution, we performed other goodness-of-fit tests, such as the goodness-of-fit index (GFI) and Bentler–Bonett normed-fit index (NFI) (Shipley, 2002; Iriondo *et al.*, 2003). GFI and NFI range between 0 and 1, with values > 0.90 indicating a good fit. For R/S and PNUE and R/S plasticity and PNUE plasticity, a total of 360 values were included (three replicates per block \times three blocks \times 40 species). For phenotypic integration, only 40 values (40 species) were included because replicates of individuals and blocks were used to estimate the percentage of correlated plastic responses among traits per species. Then, we used the generalized least-squares (GLS) method to estimate the standardized path coefficients of our model, which are equivalent to standardized partial regression coefficients (i.e. they define the relative influence of one variable on another), and its significance with multivariate Wald test. This test locates the set of path coefficients that can be considered to be zero without worsening the fit (i.e. significantly increasing the χ^2) of the model (Shipley, 2002).

Additionally, we performed explicit comparisons between invasive and native species through multigroup analysis (Shipley, 2002; Byrne, 2004; Milla, 2009), because we were aiming to distinguish whether the paths of the model differ statistically between invasive and native species. The statistical procedure was first to build a constrained model, in which all free parameters were forced to be equal across invasive and native species. This model was then compared with the outcome of the model fitted to the experimental data. Then, since a lack of fit was detected in the fully constrained multigroup model, a series of nested models were developed to detect which paths significantly improved the model when released (Shipley, 2002). For this, we removed each path of the model one at a time. The difference in the two maximum likelihood χ^2 statistics was used to test for a difference in the value of a parameter between invasive and native species after Bonferroni correction. The overall significance level of path coefficients and multigroup analysis were carried out using AMOS 5.0 software (AMOS Development Corp., Mount Pleasant, SC,

USA), whereas the rest of the SEM analyses were performed with the SEPATH procedure of the Statistica 7.0 software package (StatSoft, Inc, Tulsa, OK, USA).

Results

Fitness, mean trait values, phenotypic plasticity and phenotypic integration: invasive vs native

Invasive species displayed higher biomass and survival than native species. These differences were significant under medium nutrient supply, high nutrient supply and shade for biomass, and under shade for survival (Table 3). *R/S* ratio and PNUE significantly differed between invasive and native species. Invasive species had lower *R/S* ratios and higher PNUE values than native species across treatments (Table 3).

Invasive and native species showed similar *R/S* plasticity values across resource gradients. However, invasive species showed significantly higher PNUE plasticity than native species from low to medium nutrient supply and from shade to sun but not from medium to high nutrient supply (Table 3). Total biomass was not statistically significant when included as a covariable, meaning that observed differences in *R/S* and PNUE plasticity were not a consequence of an increase in plant size (*R/S*: low to medium nutrients, $F_{1,39} = 2.04$, $P = 0.53$; medium to high nutrients, $F_{1,39} = 0.28$, $P = 0.88$, shade to sun, $F_{1,39} = 5.31$,

$P = 0.20$; PNUE: low to medium nutrients, $F_{1,39} = 2.77$, $P = 0.46$; medium to high nutrients, $F_{1,39} = 1.49$, $P = 0.61$; shade to sun $F_{1,39} = 4.96$, $P = 0.27$). Finally, phenotypic integration tended also to be higher in invasive species, but this trend was only evident from medium to high nutrient supply (Table 3).

Structural equation modeling of phenotypic performance

Goodness-of-fit tests for SEM indicated an overall good model fit in all invasive and native models across treatments. The χ^2 test was not significant at $P > 0.05$, which implies that the covariance structure specified by each model could not be rejected. Moreover, GFI and NFI values were higher than or similar to 0.90, indicating that they provide an optimal fit compared with a null model that assumes independence among all variables (Fig. 1b–g). Importantly, we did not observe significant differences in the path coefficients (described later) between invasive and native species when the three native species invasive elsewhere were removed (i.e. probability of $\Delta ML \chi^2$ (the difference in maximum likelihood χ^2 estimates between the constrained model and the rest) between Tables 4 and S3 did not differ statistically).

From low to medium nutrient supply, invasive and native species differed in the way their increment in biomass was achieved. While PNUE plasticity had a significant and positive

Table 3 Statistical differences in root-to-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

Functional strategy	Resource level/change in resource level	Invasive	Native	<i>F</i> , <i>P</i>
<i>R/S</i>	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**
<i>R/S</i> 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	3.15 ns
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	6.74 ns
	High	0.93 ± 0.02	0.88 ± 0.03	3.18 ns
	Shade	0.93 ± 0.01	0.82 ± 0.04	14.49**

The second column shows whether traits were calculated within or between two treatments.

F- and *P*-values correspond to PERMANOVA analyses. df = 39. Mean ± standard error are also shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, nonsignificant.

Table 4 Multigroup comparison of path coefficients among invasive and native species and between resources treatments

Free parameters for which between-group equality constraint was released	Low to medium			Medium to high			Shade to sun		
	ML χ^2	Δ ML χ^2	Probability of Δ ML χ^2	ML χ^2	Δ ML χ^2	Probability of Δ ML χ^2	ML χ^2	Δ ML χ^2	Probability of Δ ML χ^2
None	204.841			210.501			90.034		
Path 1 (<i>R/S</i> 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 (<i>R/S</i> RDPI to biomass)	200.561	4.280	0.043	210.116	0.385	0.404	88.550	1.483	0.224
Path 3b (<i>R/S</i> 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 (<i>R/S</i> 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 (<i>R/S</i> RDPI 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
Error variance of survival	203.171	1.670	0.195	160.850	49.651	0.001	63.072	26.961	0.001

The first column in each section shows the maximum likelihood χ^2 estimates (ML χ^2) after constraining all free parameters to the same value. The following columns in each section 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 is given as Δ ML χ^2 , and the *P*-value indicates the probability that the release of that parameter improves the model significantly. A significant *P*-value, highlighted in bold, indicates that the relative path contribution to the model is different between invasive and native species. See Fig. 1(a) for path codes. Bonferroni-corrected *P*-value threshold, $0.05/15 = 0.003$.

direct effect on the increment of biomass for invasive species, PNUE mean did so on the biomass increment of natives (Table 4). In turn, this increment in biomass affected survival positively. Surprisingly, *R/S* and plasticity of *R/S* did not have a significant effect on fitness in any group (Fig. 1b,c). Phenotypic integration of invasive species was positively correlated with PNUE plasticity and negatively correlated with *R/S* plasticity (Table 4). Interestingly, invasive species offset to a greater extent than natives the direct negative influence of PNUE plasticity on survival (path 4b, invasive = -0.46 , native = -0.26). They achieved this by the positive indirect influence of PNUE plasticity on biomass (path 4a*path 6 = $0.61 \times 0.32 = 0.195$) plus the positive direct influence of phenotypic integration on survival (path 5b = 0.44), whereas natives had only a direct influence of PNUE on survival through biomass (path 2*path 6 = $0.40 \times 0.48 = 0.192$) (Fig. 1b,c).

Comparing medium to high nutrient supply with low to medium nutrient supply, an opposite pattern was found in relation to PNUE. Now, the increment of biomass of invasive species was positively driven by PNUE mean, and the increment of biomass of native species was positively driven by PNUE plasticity. Further, *R/S* mean was negatively related to an increment of biomass and the relative importance of this path did not differ between invasive and native species (Table 4). Here, a negative effect of *R/S* indicates a smaller *R/S* (i.e. higher *R/S*, higher above- than below-ground biomass) and has a positive effect on fitness. Moreover, the effect of phenotypic integration on the fitness of invaders highlights the complexity of observed relationships. On the one hand, high phenotypic integration values decreased survival, but, on the other, it diminished the negative

effect of *R/S* plasticity on survival, via the negative correlation between integration and *R/S* plasticity (path c3) (Fig. 1d). This complexity once again reflected the ability of invasive species to offset the direct negative effect of plasticity and integration on fitness. For native species, by contrast, phenotypic integration directly increased both survival and biomass and indirectly increased biomass through its positive correlation with PNUE plasticity (Fig. 1e).

From shade to sun, invasive and native species did not significantly differ in their path coefficients (i.e. goodness-of-fit did not improve significantly when a path coefficient was released in multigroup comparison tests) (Table 4). Lower *R/S* contributed to increased biomass and this in turn to increase survival. Increased biomass was the direct consequence of high PNUE mean values, PNUE plasticity and phenotypic integration. Also, phenotypic integration positively affected survival (Fig. 1f,g).

In summary, our results show that across resource gradients invasive and native species achieve fitness in a similar way. Trait means had a higher influence on increased biomass and survival than phenotypic plasticity and integration. In addition, increased biomass had a consistently positive effect on survival. The physiological trait and its plasticity (PNUE) had a higher positive effect on fitness than the morphological (*R/S*) one. Phenotypic integration, in turn, was positively correlated with PNUE plasticity but negatively correlated with *R/S* plasticity. Regarding differences between invasive and native species we found that, first, there was a switch in the relative importance of PNUE and PNUE plasticity for the increment of biomass across a nutrient gradient, and secondly, invasive species offset to a greater degree the few cases where plasticity and integration had a negative effect on fitness.

Discussion

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

Multiple strategies promote fitness and reduce maladaptive responses

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

Although less well studied, maladaptive plastic responses can be common (van Kleunen & Fischer, 2005; Valladares *et al.*, 2007). For instance, a negative influence of plasticity on survival was found in four Iberian tree species grown along a light gradient (Sánchez-Gómez *et al.*, 2006). However, it is still unclear how relevant maladaptive responses are to species performance and how these responses translate to community dynamics (Miner *et al.*, 2005). In our study, maladaptive responses were important but they were offset by the positive effect of other aspects of the functional strategy, where invasive species did better. Instead of studying maladaptive responses in isolation, we encourage the application of multivariate approaches such as the one followed here to test how the variation in the ratio between adaptive and maladaptive responses affects overall plant fitness and hence their invasive potential.

Little support for different functional strategies between invasive and native species along resource gradients

Invasive and native species differed in the relative importance of PNUE and PNUE plasticity along the nutrient gradient. Only invasive species support the hypothesis that higher plasticity of traits associated with resource use efficiency (i.e. PNUE

plasticity) are crucial for achieving higher biomass from limiting to nonlimiting resource availabilities (i.e. low to medium nutrient), as Funk (2008) found. However, under high resource availability (medium to high nutrient), high PNUE was more important for achieving high biomass. We did not find evidence to support the hypothesis of the higher relative importance of PNUE plasticity vs PNUE mean values for invasive species from shade to sun, in contrast to the findings of other studies (Poorter, 1999; Sánchez-Gómez *et al.*, 2006). These discrepancies may be the result of the relatively high light intensity of our shade treatment (20% of full sunlight) as compared with those used in these studies (6–10%).

Surprisingly, morphological plasticity, represented by R/S plasticity, did not influence fitness. This contrasts with other studies which reported that plasticity of morphological traits, such as R/S , SLA, or LAR, is usually involved in adaptive responses to light or nutrient shifts (Valladares *et al.*, 2000; Dudley, 2004; Hodge, 2004; Funk, 2008). Our results are perhaps caused by differences in timescale, as morphological plasticity has a slower response time than physiological plasticity (Funk *et al.*, 2007). Given that we measured fitness after a short time (6 months), it might be more influenced by the more dynamic physiological plasticity. The implications for plant performance of displaying a more or less dynamic plasticity are still not clear (Funk *et al.*, 2007), but presumably the future costs associated with a more dynamic plasticity (such as PNUE plasticity) will be less than the costs associated with a less dynamic plasticity (such as R/S plasticity). For instance, high R/S plasticity to shade can generate a maladapted phenotype to a future drought (Valladares *et al.*, 2007). Another explanation for these results might be that our resource gradients were not wide enough to elicit a significant R/S variation in 6 months. However, this latter explanation seems less likely because analogous studies with similar growth lengths and resource gradients have obtained significant results (Sánchez-Gómez *et al.*, 2006; Funk, 2008).

The importance of phenotypic integration for invasiveness: new evidence

Phenotypic integration also followed a pattern of adaptive, non-adaptive and maladaptive mix. Thus, their relative importance for invasiveness can be considered similar to that of plasticity but lower than trait means. Interestingly, phenotypic integration primarily promoted survival, which may be important for establishment success of invasive taxa. However, our results suggest that the most important role for invasiveness was the consistent positive effect on the expression of adaptive PNUE plasticity. In this sense, we support, but only partially, the notion that phenotypic integration may constrain phenotypic plasticity (see suggestions by Gianoli, 2004; Gianoli & Palacio-López, 2009). When significant, morphological plasticity (R/S ratio) was negatively correlated with phenotypic integration. By contrast, physiological plasticity (PNUE) was not. Our discrepancy with Gianoli & Palacio-López (2009) may be a result of the fact that they only correlated phenotypic integration to morphological plasticity.

We hypothesize that a more integrated phenotype responds to environmental variation with traits of faster plastic responses because highly dynamic plasticity does not typically entail changes in other traits at higher levels of organization. By contrast, a plastic response in a morphological trait such as R/S plasticity, which determines key aspects of plant architecture, may entail a plastic response in other traits at lower levels of organization in a cascade effect.

This link between integration and highly dynamic plasticity may be important for invasiveness because exotic plants render faster adaptive plastic responses with a lower risk of mortality.

Conclusion

Previous studies have shown the importance of particular traits and trait plasticity as determinants of invasiveness. But their relative importance was not explored because they were not explicitly studied in combination. Besides, the role of phenotypic integration in invasiveness remained unexplored. According to our multivariate models, we conclude that fitness in both invasive and native species is jointly determined by trait mean values, their phenotypic plasticity and phenotypic integration, following a fitness hierarchy. Invasive species had higher fitness than natives because they showed higher PNUE and lower R/S , which was positively associated with fitness along gradients; they showed higher physiological plasticity, which was also associated with higher biomass; and they offset more the cases when plasticity and integration had a negative influence on fitness. Although the relative contribution of these three components of the functional strategy to invasiveness may be modulated by other factors associated with human activities (Pyšek *et al.*, 2009), multivariate approaches such as the one followed here are very promising as a means of disentangling which factors are promoting plant fitness and hence the invasion potential of exotic species.

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Supporting Information

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

Fig. S1 Allometric relationship between root and shoot (R/S) and between maximum photosynthetic rate and leaf nitrogen content per unit of area (PNUE) for invasive and native species along resource gradients.

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

Table S2 Trait loadings extracted by a principal component analysis (PCA) in order to calculate phenotypic integration

Table S3 Multigroup comparison of path coefficients among invasive and native species and between resources treatments, excluding the three native species invasive elsewhere.

Notes S1 UTM coordinates where seeds were obtained.

Notes S2 Detailed description of the traits measured.

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