

# Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity

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## Summary

**1.** Plastic responses to spatiotemporal environmental variation strongly influence species distribution, with widespread species expected to have high phenotypic plasticity. Theoretically, high phenotypic plasticity has been linked to plant invasiveness because it facilitates colonization and rapid spreading over large and environmentally heterogeneous new areas.

**2.** To determine the importance of phenotypic plasticity for plant invasiveness, we compare well-known exotic invasive species with widespread native congeners. First, we characterized the phenotype of 20 invasive–native ecologically and phylogenetically related pairs from the Mediterranean region by measuring 20 different traits involved in resource acquisition, plant competition ability and stress tolerance. Second, we estimated their plasticity across nutrient and light gradients.

**3.** On average, invasive species had greater capacity for carbon gain and enhanced performance over a range of limiting to saturating resource availabilities than natives. However, both groups responded to environmental variations with high albeit similar levels of trait plasticity. Therefore, contrary to the theory, the extent of phenotypic plasticity was not significantly higher for invasive plants.

**4.** We argue that the combination of studying mean values of a trait with its plasticity can render insightful conclusions on functional comparisons of species such as those exploring the performance of species coexisting in heterogeneous and changing environments.

**Key-words:** ecophysiological traits, exotic species, light, nutrient, phylogenetic conservatism, phylogenetically independent contrast, resource-use efficiency

## Introduction

Linking phenotypic plasticity to invasiveness of exotic species is an important topic in the study of biological invasion (Richards *et al.* 2006; Funk 2008; Hulme 2008). Phenotypic plasticity, measured as the ability to express different phenotypes in different environments (Sultan 1995; Valladares *et al.* 2000; Pigliucci 2001; West-Eberhard 2003), has been widely predicted to be a key trait for explaining why exotic invasive species are so successful in their recipient communities (e.g. Williams, Mack & Black 1995; Sakai *et al.* 2001; Sexton, McKay & Sala 2002; Niinemets, Valladares & Celemans 2003; Funk 2008). Theoretically, high plasticity is likely to influence the potential invasiveness of species

because it may enable them to express advantageous phenotypes over a broad range of environments (detailed in Mate-sanz, Gianoli & Valladares 2010), potentially enhancing their ecological success and their impact across ecosystems (Alpert, Bone & Holzappel 2000; Daehler 2003; Hulme 2008). For instance, it has been postulated that plasticity would reduce the risk of stochastic local extinction after the arrival of a few genotypes and augment the likelihood of species to become invasive under environmental conditions to which they were not preadapted (Sultan 2001; Ghalambor *et al.* 2007; Bossdorf, Lipowsky & Prati 2008). High plasticity can be due either to an adaptive strategy to cope with spatiotemporal resource fluctuation in the native region (Williams, Mack & Black 1995; Atkin *et al.* 2005) or to rapid evolution in novel sites after colonization (Agrawal 2001; Yeh & Price 2004). Although it still remains to be tested, the higher the capacity to vary a broad number of morphological and ecophysiological traits the higher the

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likelihood of invaders to be preadapted to invasion (Hulme 2008), especially under low resource environments (Davis, Grime & Thompson 2000).

Richards *et al.* (2006) classified invasive species into three categories according to the fitness outcome owing to plasticity as compared with a control group of native/noninvasive species: two main categories 'Jack-of-all-trades' and 'Master-of-some', and a third category 'Jack-and-Master', which is a combination of the first two. Under the 'Jack-of-all-trades' strategy, successful invasive species are hypothesized to have homeostasis of fitness (i.e. fitness remains constant along a resource gradient), because they are better able to maintain higher fitness in unfavourable environments. Several studies have shown evidence for this strategy. For example, invasive species (two trees, one shrub and two grasses) in Hawaiian Islands varied their specific leaf area (SLA) to maximize light capture in response to a decrease in light availability (Funk 2008). In response to increased temperature, *Ailanthus altissima* and *Acer platanoides*, two widespread invasive tree species, shifted their biomass allocation from transpiring tissues to roots and water-transporting tissues (Saümel 2006). High plasticity in root/shoot ratio resulted in enhanced water uptake under drought conditions in species such as *Alternanthera philoxeroides* and *Taraxacum officinale* (Brock & Galen 2005; Geng *et al.* 2006). In contrast to the 'Jack-of-all-trades' strategy, the 'Master-of-some' strategy encompasses those successful invasive species able to obtain higher fitness in response to an increase in resources availability via phenotypic plasticity. Finally, 'Jack-and-Master' strategy includes those successful invasive species that are better able to maintain and to increase fitness in unfavourable and favourable environments, respectively.

Although arguments in favour of linking phenotypic plasticity to plant invasion seem reasonable, no general pattern between phenotypic plasticity and invasiveness has emerged so far. While some studies support higher plasticity of invaders (Niinemets, Valladares & Ceulemans 2003; Schumacher *et al.* 2009; Davidson, Jennions & Nicotra 2011), others do not (Bossdorf *et al.* 2005; Peperkorn, Werner & Beyschlag 2005; Funk 2008). Nevertheless and despite the fact that trait plasticity and trait mean value covary, only trait plasticity has been taken into account (but see Stinchcombe & Schmitt 2006; Callahan & Pigliucci 2002 for selection analyses of plasticity). With the same adaptive extent of phenotypic plasticity, one species may display higher fitness compared with another if the value of a given trait that account for fitness is significantly higher in the former. Accordingly, even low plasticity may be advantageous for the former species if the difference in the mean value of this trait is proportionally higher than the difference in plasticity displayed between the two species. This may explain why some studies have found that invasive species outperform native species even when the two have the same level of plasticity (e.g. Peperkorn, Werner & Beyschlag 2005; Funk 2008). Therefore, if we want to assess the importance of phenotypic plasticity to the invasiveness of exotic species, the question is not only how plastic an invasive species is, but how is the trait mean value related to fitness.

The aim of this study was to explore phenotypic plasticity in exotic invasive species using a common garden experiment. Twenty phylogenetically related pairs of invasive-native species covering a diversity of growth forms (trees, shrubs, perennial and annual herbs) that co-occur in Mediterranean ecosystems were compared measuring 20 different traits in a common garden experiment across one nutrient and one light gradient. Specifically, we ask (i) whether exotic invasive species and native species differ in their trait mean values, (ii) whether exotic invasive species have higher levels of plasticity than native species, (iii) whether differences of particular trait plasticity and overall mean trait plasticity between both groups are dependent on the level of the resource gradient considered or not.

Studies at both species- and genotype-level are useful approaches to explore the relationship between plasticity and invasiveness (Richards *et al.* 2006). Genotype-level approaches provide a precise study of plasticity, its mechanisms and its evolutionary potential (Sultan 2000). However, the species-level approach allows for broader generalizations if many species and traits are included (Schlaepfer *et al.* 2010) and for the development of risk-assessment protocols because species level is the most frequent taxonomic level for coping with invasions in practice (van Kleunen, Weber & Fischer 2010). Also, multispecies comparison allows us to explore whether plasticity is phylogenetically conserved (Kembel & Cahill 2005), which may increase our capacity to predict potential invasiveness from phylogenetic information of the species. Despite their importance, studies involving multispecies comparison are scarce and most functional studies of invasive organisms are restricted to only a few species (but see Schlaepfer *et al.* 2010) and a limited number of traits.

## Materials and methods

### SPECIES SELECTION

From January to October 2005 and 2006, plants from twenty exotic and twenty native species were grown from seeds in the Botanical Garden of the University of Alcalá (40°28'N, 3°22'W, 588 m). The exotic species set was comprised of introduced species clearly invasive in the Iberian Peninsula (*sensu* Pyšek *et al.* 2004), local dominants in some native ecosystems (Valéry *et al.* 2008) and species with potential impact on native ecosystems (transformer species, *sensu* Richardson *et al.* 2000). Overall, they represent the broad range of taxonomy, invaded habitats (woodlands, scrublands, grasslands and riparian areas) and growth forms (annual and biannual herbs, shrubs and trees) of invasive exotic species in the Iberian Peninsula (Sanz Elorza, Dana Sanchez & Sobrino Vesperinas 2004). Nomenclature follows the Iberian Flora (Castroviejo 1986–2008), which is in agreement with the Missouri Botanical Garden, VAST nomenclatural database (W3Tropicos, <http://www.tropicos.org/>).

When assessing differences between invasive and native species, it is important to take phylogenetically independent contrast controls into account as well as to ensure that within pairs both species occur in similar ecosystems (Lambdon & Hulme 2006). Thus, we paired each invader with one closely related native species based on the following phylogenetic and ecological guidelines: (i) the native species had to belong to the same family as the invasive species (that was

**Table 1.** Variables and descriptions of the traits measured. Effective quantum yield, photochemical (qP) and nonphotochemical quenchings (NPQ) and electronic transportation rate were measured at saturating light level (1900  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ )

Variable	Description	Units
Plant-level traits		
LWR	Leaf weight ratio	$\text{g leaf g}^{-1} \text{ plant}$
SWR	Stem weight ratio	$\text{g stem g}^{-1} \text{ plant}$
RWR	Root weight ratio	$\text{g root g}^{-1} \text{ plant}$
LAR	Leaf area ratio	$\text{m}^2 \text{ leaf kg}^{-1} \text{ plant}$
Leaf-level traits		
$A_{\text{max}}$	Maximum photosynthetic rate at saturating light per unit area	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
iWUE	Instantaneous water-use efficiency	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$
PNUE	Photosynthetic nitrogen-use efficiency	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$
$N_{\text{area}}$	Leaf nitrogen content per unit area	$\text{g N m}^{-2} \text{ leaf}$
$N_{\text{mass}}$	Leaf nitrogen concentration	$\text{mg N g}^{-1} \text{ leaf}$
SLA	Specific leaf area	$\text{cm}^2 \text{ leaf g}^{-1} \text{ leaf}$
$R_{\text{dark}}$	Plant respiration	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
Curvature factor ( $\Theta$ )	Light curve convexity	–
Compensation point ( $\Gamma$ )	Light compensation point	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
Saturation point ( $I_c$ )	Light saturation point	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
qP <sub>1900</sub>	Photochemical quenching	–
qN <sub>1900</sub>	NPQ associated with radiant energy dissipation	–
NPQ <sub>1900</sub>	NPQ associated with nonradiant energy dissipation	–
$F_v/F_m$	Ratio of variable to maximum fluorescence	–
$\Phi_{\text{PSII1900}}$	Effective quantum yield of PSII	$\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$
ETR <sub>1900</sub>	Electron transport rate	$\mu\text{mol e}^- \text{ m}^{-2} \text{ leaf s}^{-1}$
Fitness-related variables		
Survival	Percentage of survival during growth	%
Total biomass	Total above- and below-ground biomass	$\text{g plant}$

achieved in 17 of 20 pairs), (ii) they had to have the same growth form (achieved in all pairs except number 15 and 16 (See Table S1 in Supporting Information), in which invasive species were trees and natives shrubs), (iii) they had to coexist in the same habitat-type in the Iberian Peninsula and the same successional community stage and (iv) they had to be recorded as co-occurring at least once in Spain. 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 rare or with endangered status were excluded.

#### EXPERIMENTAL DESIGN

Several resource concurrent gradients influence plastic responses of plants (Portsmouth & Niinemets 2007). However, to make useful predictions, we need to quantify potentially adaptive plastic traits to one resource at a time using a realistic resource gradient (e.g. Poorter 1999; Quero *et al.* 2006; Sánchez-Gómez, Valladares & Zavala 2006; Funk 2008), despite the limitations of this approach (Hulme 2008). Accordingly, we designed a nonfactorial experiment with two resource gradients using two different greenhouses owing to logistical limitations: Nutrient gradient (low-medium-high) under the same sunlight conditions and light gradient (shade-sun) under the same medium nutrient conditions. Within the 'nutrient greenhouse' radiation was kept constant at 50% full radiation ( $950\text{--}1050 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and light quality red/far red ratio ( $R : fR$ ) = 1. Within 'light greenhouse' plants were subjected to medium nutrient growth environment with 'shade' being 20% of full sunlight radiation ( $350\text{--}500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and light quality modified to  $R : fR = 0.8$ , which is the most common shade under Mediterranean ecosystems (Valladares 2004). Although we did not use low light levels (e.g. 1–15% full radiation) that will potentially show the nonlinear plant

responses to light variation (Poorter 1999), this design enabled us to reasonably frame our hypothesis across nutrient and light availabilities where exotic species invade in Spanish Mediterranean ecosystems.

Half of the species pair were grown during 2005 and the other half during 2006 (see Table S1). In each year, 144 plants per species were grown from seeds in individual 1 L pots (QP 12T/18; PROJAR, Spain) with vermiculite (0–3 mm grain,  $80\text{--}100 \text{ kg m}^{-3}$ ; PROJAR). Seeds were obtained from commercial supply or field collection and sown in March of the corresponding year and grown for a full growing season. In both cases, seeds came from locations where the exotic species are clearly invasive. For commercial supply, seeds were certified to come from one single location. For field collection, we collected seeds from 15 to 20 haphazardly chosen plants within one population. Population delimitation was according to Schlaepfer *et al.* (2010) criteria.

In each greenhouse, plants were divided into three different blocks (12 plants per block\*species\*treatment) to control for possible variation in measurements because of microclimatic gradients. In addition, plants were randomly positioned in their block and rotated every month. We fertilized plants at the beginning of the experiment with a Plantacote mix 6-month slow-release fertilizer 14-9-15 N-P-K, (Aglukon Spezialdünger GMBH & Co.KG, Dusseldorf, Germany). We used a slow-release fertilizer to ensure that plants had available nitrogen throughout the experiment. The main nitrogen compound was ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) (85%). Plants received one dose, over the 6 months, equivalent to  $0.010 \text{ g N}$  in the low nutrient level,  $0.085 \text{ g N}$  in the medium nutrient level and  $0.245 \text{ g N}$  in the high nutrient level. Pure vermiculite was used as substrate in the experiments 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 photosynthetic photon

flux density (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, NB, USA). Mean daily PPFD (400–700 nm) over the summer was  $41 \text{ mol m}^{-2} \text{ day}^{-1}$ , which is equivalent to full sunlight.

#### PHENOTYPIC TRAITS

We measured 20 variables related to plant- and leaf-level traits (see Table 1 for abbreviations). Traits were selected because of their functional significance for resource acquisition [e.g. high leaf area ratio (LAR) and weight ratio of root (RWR) are associated with light and nutrient acquisition, respectively], plant competition (e.g. high rate of maximum photosynthesis ( $A_{\text{max}}$ ) and  $F_v/F_m$  are associated with fast growth and good physiological status) and stress tolerance [e.g. high photosynthetic nitrogen-use efficiency (PNUE) is associated with high plant performance in nitrogen-limited environments and high SLA in light-limited environments]. Finally, we measured survival ( $n = 36$  per species\*treatment) and total biomass (above- plus below-ground biomass) after 6 months since germination as surrogates of fitness. These variables are commonly used in short-term studies of perennial species when reproductive measures are difficult to obtain (Sultan *et al.* 1998; Funk 2008).

Plant-level traits were recorded for nine replicate plants per treatment and species (plants were arranged in three blocks). Each individual plant was separated into leaves, stems and roots, oven-dried at  $60 \text{ }^\circ\text{C}$  for 3 days and weighed to calculate weight ratio of leaf (LWR), stem (SWR) and root (RWR) per total biomass. Before oven-drying the material, leaf area of each whole individual was measured using a Delta-T leaf area meter device (Delta-T devices, Cambridge), to calculate leaf area ratio (LAR = leaf area/plant dry mass) and specific leaf area (SLA = leaf area/leaf dry mass).

Because the selected plant-level traits vary with ontogeny (Evans 1972; Poorter 1999), we used a nondestructive method for measuring plant volume over time that partially corrected for possible ontogenetic development drift between treatments (e.g. usually plants exhibit faster development under higher nutrient availability). To satisfy both needs, we first followed the growth dynamics measuring plant volume as a function of height and crown cover for each species and nutrient treatment in each month. For this, we used the semi-sphere equation ( $V = (2/3) \times \pi \times r^2 \times H$ ) where  $H$  is height and  $r$  is the mean radius ( $r$ ) of plant cover (maximum radius + minimum radius/2). Later, during August, we fitted for the mean plant volume of invasive and native species a sigmoidal function to predict their volume increase. Results revealed that plants growing under the high nutrient treatment developed faster (i.e. ontogenetic drift). Therefore, we partly correct for differences on ontogeny across treatments by collecting plants grown in the higher nutrient treatment 1 month before the end of the experiment (October of 2005 or 2006) (see Appendix S1). This procedure also ensured that the harvesting period was short enough to avoid an effect on the results.

Leaf-level traits were measured using a LI-6400 portable photosynthesis system with a fluorescence chamber (LI-COR) in one mid-height undamaged fully expanded leaf ( $n = 3$  plants randomly selected per species and treatment). We constructed light response curves at 10 light intensities of PAR following the order 0, 800, 1100, 1500, 1900, 500, 250, 150, 100, 50 and  $0 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  and with the following constant conditions:  $\text{CO}_2$  concentration 400 ppm, flow  $400 \text{ cm}^3 \text{ min}^{-1}$ , air humidity 40–60% and block temperature  $25 \text{ }^\circ\text{C}$ . First, we adapted the leaf to dark for 30 min to measure respiration

( $R_{\text{dark}}$ ). Then, the leaf was irradiated with saturating and noninhibitory light ( $800 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) for 10 min to be sure that plants were photosynthetically active. We then changed light intensity and recorded maximum photosynthetic rate ( $A_{\text{max}}$ ) at each light level when it was stable (i.e. every 3 min on average). At maximum light intensity ( $1900 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $T$ ) was also recorded to subsequently calculate instantaneous water-use efficiency ( $i\text{WUE} = A_{\text{max}}/T$ ). Instantaneous WUE was calculated instead of intrinsic WUE =  $A_{\text{max}}/g_s$  because we were interested in the gas exchange ratio between carbon acquisition and water release. From dark to maximum light intensity, we measured a set of fluorescence parameters [ $F_v/F_m$ ,  $\Phi_{\text{PSII}}$ ,  $qP$ ,  $qN$ , nonphotochemical quenching (NPQ) and ETR] (see Appendix S2). Additionally, we selected three random plants per species and treatment to measure  $A_{\text{max}}$  and WUE in a total of six plants per species and treatment.

We obtained photosynthetic parameters from light response curves using Photosyn Assistant software version 1.1.1 (Richard Parsons, Dundee, UK). This software models the photosynthetic response of leaves to variation in light level by a rectangular hyperbola following the quadratic equation presented by Chartier & Prioul (1976), where the light compensation point ( $\Gamma$ ) is estimated from intercept to  $x$ -axis, the light saturation point ( $I_c$ ) is the light level at which the leaf reaches its maximal photosynthetic capacity and the convexity light curve factor ( $\Theta$ ) describes the progressive rate of bending between the linear gradient and the maximum value.

We measured organic leaf nitrogen concentration per mass ( $N_{\text{mass}}$ ) and per area ( $N_{\text{area}}$ ) at Nutrilab (University Rey Juan Carlos, Móstoles, Madrid, Spain) with segmented flux autoanalyzer (S-F.A.S. Skalar San ++), after digestion with  $\text{H}_2\text{SO}_4$  and  $\text{Cu-KSO}_4$ , which converts all organic nitrogen into ammonium ( $\text{NH}_4^+$ -N). Previously, leaves of each species and treatment had been pooled within blocks and ground in a Culatti mill to 1 mm particle size. After that,  $N_{\text{area}}$  was calculated by dividing N leaf content by the leaf area mean and PNUE as the division of  $A_{\text{max}}$  by  $N_{\text{area}}$ . Mean values for each trait and species are shown in Table S2.

#### PHENOTYPIC PLASTICITY

We calculated trait variation for each plant species with the plasticity index (PI) created by Valladares *et al.* (2000).

$$\text{PI} = \frac{\text{Mean}(\text{env1}) - \text{Mean}(\text{env2})}{\text{Max}(\text{Mean}(\text{env1}), \text{Mean}(\text{env2}))}$$

$\text{Mean}(\text{env1})$  and  $\text{Mean}(\text{env2})$  are the mean values of a given trait for one species in the environment 1 and 2, representing the mathematical expression of a reaction norm.  $\text{Max}(\text{Mean}(\text{env1}), \text{Mean}(\text{env2}))$  serves to standardize the index, which ranges from zero (no plasticity) to one (maximum plasticity). PI also indicates the direction of the change. For instance, a negative PI value indicates that the mean value of a given trait is higher under environment 2. We preferred PI to other published plasticity indices (see list of plasticity indices in Valladares, Sanchez-Gomez & Zavala 2006) because it is the index that better reflects a reaction norm and it is not sensitive to differences in variance between two samples. PI was calculated for each trait and species along the two resource gradients. We also calculated the mean plant-level and leaf-level plasticity, as well as the overall mean plasticity.

Finally, it must be noted that we calculated PI for shade to sun under light gradient (Sh-S) and for each experimental resource

change under nutrient gradient (i.e. low-to-medium, medium-to-high nutrient level (L–M, M–H)), instead of for the extreme of the gradient (i.e. low–high nutrient level L–H), to relate PI values to ‘Jack-of-all-trades, Master-of-some’ framework.

#### STATISTICAL ANALYSIS

We evaluated the effect of invasiveness (two levels, invasive vs. native), block (three levels) and phylogenetic distance within pairs on phenotypic traits, on different trait-plasticity scales (each trait plasticity, mean plant-level and leaf-level plasticity, and overall mean plasticity) and, finally, on fitness in the two resource gradients using a nonparametric analysis of variance (PERMANOVA, Anderson 2001, 2005). We selected a PERMANOVA approach because it permits pairwise comparison at different phylogenetic levels. This type of analysis was also selected because it does not make assumptions of normality or homoscedasticity of the data and its residuals. We initially performed an analysis with all 20 phenotypic traits included, considering invasive/native status and nutrient/light levels as fixed factors, block as a random factor and phylogenetic distance within pairs as a covariable. Next, a series of models including one trait at a time as the dependent variable were performed to search for the possible differences found in the first model including all variables. The same statistical procedure was performed for the plasticity of the 20 measured traits, for the mean plant-level and leaf-level plasticity, as well as for the overall mean plasticity. However, we took a slightly different approach when we analysed each trait plasticity separately. Because plant size can directly influence biomass partitioning and thus plant-level trait plasticity (Poorter 1999; Funk 2008), we also included biomass as a covariable to test whether observed plasticity was a mechanistic consequence of an increase in plant size (apparent plasticity *sensu* Dudley 2004; Hulme 2008) or a plastic strategy *per se*. Analyses were conducted to compare data from L to M nutrient levels and M to H nutrient levels and from Sh to S light levels. In all cases, significant results between invasive and native pairwise comparisons and post hoc comparisons were estimated from 9999 permutations using Bray–Curtis dissimilarity, which measures the distance between the trait values that remains unique to one group (invasive or native) divided over the trait values common to both groups. 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).

#### PHYLOGENETIC PATTERN OF PLASTICITY

We investigated phylogenetic conservatism in plasticity at different taxonomic levels by implementing the phylogenetic node-dated tree of our invasive and native species set and plasticity values (mean plant-level and leaf-level plasticity, overall mean plasticity) into the analysis of traits (AOT), module of Phylocom package (Webb, Ackerly & Kembel 2008). However, we did not correct for ontogenetic drift here as we used mean plasticity values. To perform the analyses, we first built a pruned phylogenetic tree with the study species as terminal tips using the maximally resolved seed plant tree available in Phylomatic (<http://www.phylodiversity.net/phyloomatic/>). Next, we calibrated the resulting tree by dating the nodes with the Branch Length ADJUSTment function (BLADJ), another module of Phylocom, on the basis of clade age estimation of Wikstrom, Savolainen & Chase (2001). Once we obtained a node-dated tree, calibrated in mil-

lions of years, we introduced plasticity values into the AOT to calculate divergence–convergence degree at each internal node of the tree. The standard deviation between trait means of daughter nodes was used as a proxy of the degree of divergence at the focal node (i.e. divergence size). Significance of divergence size was estimated by 20 000 randomly permuting trait values across the tips of the phylogeny at a  $P$ -value  $< 0.05$ .

Furthermore, we dealt with polytomies in the input tree (Butler & King 2004) by randomly generating 100 fully resolved trees using MESQUITE (Maddison & Maddison 2009). We then re-sampled 50 of the 100 fully resolved trees randomly and ran the analyses described above again separately for each of those 50 trees. None of the results described in the following section changed with input tree, which supports the robustness of the analyses above to phylogenetic uncertainty.

## Results

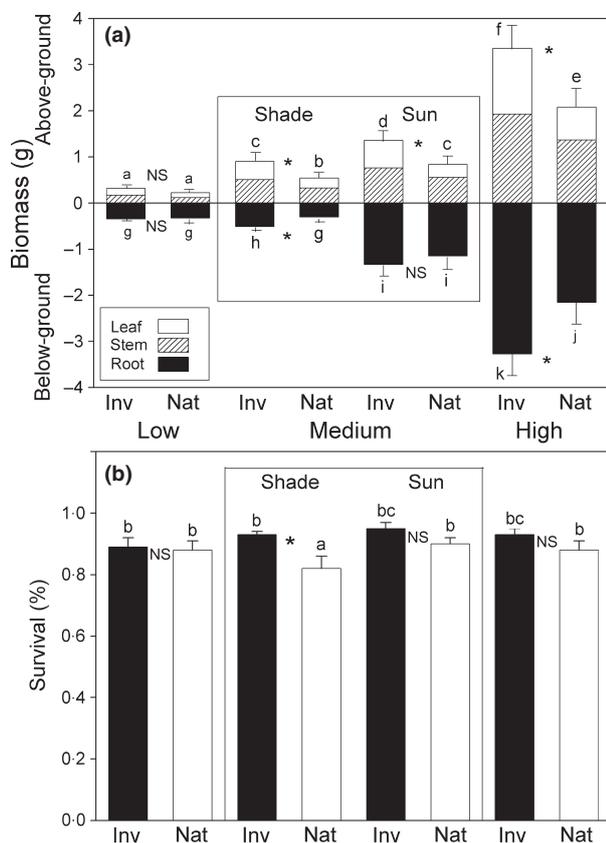
#### MEAN PHENOTYPIC VALUES AND PERFORMANCE

Results of the PERMANOVA indicated significant differences from plant- to leaf-level traits between invasive species and natives (Table 2, Table S2). Plant allometry was similar in both groups, except that invasive species assigned more resources to above-ground plant construction (higher SWR and lower RWR). Invasive species showed the same maximum photosynthetic rate ( $A_{\max}$ ) as natives but surprisingly their nitrogen leaf concentration per mass and per area was lower ( $N_{\text{mass}}$ ,  $N_{\text{area}}$ ). Thus, the PNUE was higher for invasive species (Table 2). Despite both groups exhibiting the same maximum photosynthetic rates, invasive species reached this value at lower light intensities (light saturation point,  $I_c$ ) and with faster light saturation (light curve convexity,  $\Theta$ ). In addition, invasive species had lower instantaneous water-use efficiency (iWUE) than natives (Table 2). The different ways of leaf photo-protection of invasive and native species led to convergent results in the physiological status of the plants (i.e. no significant differences were found in  $F_v/F_m$ ). Invasive species protected their photosynthetic machinery against an excess of light through higher NPQ associated with a greater number of rich-carbon photo-protective pigments (e.g. xanthophylls), whereas native species used greater amounts of chlorophyll (photochemical quenching, qP) (Table 2). In summary, differences on the light curve shape and the ability to produce photo-protective pigments based on rich carbon compounds reflected the fact that invasive species possessed a fast growth strategy because of faster carbon acquisition. Congruent with these results, invasive species had higher biomass than natives at medium and high nutrient levels but not at low nutrient level (Fig. 1). In the case of the light gradient, greater light availability had a positive effect on biomass for both groups, but invasive species always exhibited higher above-ground biomass for both light treatments (shade and sun) and higher total biomass than natives under low light availability (shade). PERMANOVA results revealed that plant survival did not differ between invasive and native species along the nitrogen gradient [ $F(\text{nutrient})_{2,119} = 1.17$ ,  $P = 0.76$ ;  $F(I/N)_{1,119} = 0.89$ ,  $P = 0.83$ ;  $F(\text{nutrient}*I/N)_{1,119} =$

**Table 2.** Mean  $\pm$  SE plant-level and leaf-level traits and their plasticities across nutrient and light gradients for twenty pairs of phylogenetically related invasive and native species across light and nutrient treatments. Woody pairs are  $n = 11$ , and herbaceous pairs are  $n = 9$ , d.f. = 39. Significantly higher values ( $P < 0.05$  or below) across invasive/native pairs, on the basis of a PERMANOVA analysis, are denoted by bold font. PI is the plasticity index of Valladares *et al.* (2000). Units do not apply to PI values. Mean  $\pm$  SE trait values for each species are supplied in Table S2

Variable	Trait mean value		PI low-to-medium nutrients		PI medium-to-high nutrients		PI shade to sun	
	Invasive	Native	Invasive	Native	Invasive	Native	Invasive	Native
<b>Plant-level traits</b>								
LWR (g)	0.21 $\pm$ 0.02	0.21 $\pm$ 0.01	0.09 $\pm$ 0.08	0.08 $\pm$ 0.07	-0.01 $\pm$ 0.06	-0.11 $\pm$ 0.04	0.33 $\pm$ 0.06	0.44 $\pm$ 0.07
SWR (g)	<b>0.28</b> $\pm$ 0.02	0.24 $\pm$ 0.02	-0.03 $\pm$ 0.08	-0.01 $\pm$ 0.08	-0.14 $\pm$ 0.03	-0.11 $\pm$ 0.06	0.23 $\pm$ 0.07	0.22 $\pm$ 0.10
RWR (g)	0.50 $\pm$ 0.02	<b>0.55</b> $\pm$ 0.02	0.02 $\pm$ 0.02	0.07 $\pm$ 0.04	-0.08 $\pm$ 0.02	-0.09 $\pm$ 0.02	-0.34 $\pm$ 0.05	-0.31 $\pm$ 0.03
LAR (m <sup>2</sup> kg <sup>-1</sup> )	3.00 $\pm$ 0.56	3.36 $\pm$ 0.63	-0.14 $\pm$ 0.08	-0.11 $\pm$ 0.07	<b>-0.18</b> $\pm$ 0.07	0.01 $\pm$ 0.05	0.60 $\pm$ 0.06	0.70 $\pm$ 0.04
<b>Leaf-level traits</b>								
$A_{\max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	8.97 $\pm$ 0.40	8.88 $\pm$ 0.41	<b>-0.27</b> $\pm$ 0.07	-0.09 $\pm$ 0.07	-0.13 $\pm$ 0.04	-0.21 $\pm$ 0.04	-0.15 $\pm$ 0.05	-0.12 $\pm$ 0.06
iWUE ( $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ )	2.73 $\pm$ 0.08	<b>3.33</b> $\pm$ 0.22	-0.14 $\pm$ 0.06	-0.07 $\pm$ 0.05	-0.08 $\pm$ 0.02	-0.02 $\pm$ 0.06	-0.17 $\pm$ 0.03	-0.16 $\pm$ 0.06
PNUE ( $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$ )	<b>164.07</b> $\pm$ 10.46	136.44 $\pm$ 10.01	<b>-0.31</b> $\pm$ 0.08	-0.08 $\pm$ 0.07	-0.12 $\pm$ 0.03	-0.17 $\pm$ 0.05	<b>-0.46</b> $\pm$ 0.05	-0.23 $\pm$ 0.08
$N_{\text{area}}$ (g N m <sup>-2</sup> )	0.73 $\pm$ 0.06	<b>0.86</b> $\pm$ 0.07	-0.66 $\pm$ 0.11	-0.45 $\pm$ 0.11	-0.50 $\pm$ 0.06	-0.48 $\pm$ 0.09	0.07 $\pm$ 0.05	<b>-0.21</b> $\pm$ 0.08
$N_{\text{mass}}$ (mg N g <sup>-1</sup> )	15.34 $\pm$ 0.78	<b>18.71</b> $\pm$ 0.83	0.04 $\pm$ 0.02	-0.07 $\pm$ 0.03	-0.02 $\pm$ 0.03	0.04 $\pm$ 0.04	0.31 $\pm$ 0.05	0.24 $\pm$ 0.06
SLA (cm <sup>2</sup> g <sup>-1</sup> )	210.7 $\pm$ 18.9	218.2 $\pm$ 16.7	0.02 $\pm$ 0.04	0.07 $\pm$ 0.05	0.10 $\pm$ 0.05	0.06 $\pm$ 0.04	0.39 $\pm$ 0.06	0.46 $\pm$ 0.03
$R_{\text{dark}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	-1.30 $\pm$ 0.09	-1.36 $\pm$ 0.08	-0.01 $\pm$ 0.09	-0.07 $\pm$ 0.07	-0.02 $\pm$ 0.08	-0.11 $\pm$ 0.08	-0.17 $\pm$ 0.10	-0.07 $\pm$ 0.05
Curvature factor ( $\Theta$ )	<b>0.61</b> $\pm$ 0.02	0.49 $\pm$ 0.03	-0.09 $\pm$ 0.09	-0.03 $\pm$ 0.11	0.02 $\pm$ 0.08	0.10 $\pm$ 0.09	<b>-0.05</b> $\pm$ 0.04	0.17 $\pm$ 0.07
Compensation point ( $\Gamma$ ) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	30.6 $\pm$ 3.2	36.1 $\pm$ 4.6	0.14 $\pm$ 0.13	0.04 $\pm$ 0.10	0.03 $\pm$ 0.10	0.05 $\pm$ 0.09	-0.29 $\pm$ 0.10	-0.20 $\pm$ 0.11
Saturation point ( $I_c$ ) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	226.9 $\pm$ 9.8	<b>269.3</b> $\pm$ 14.1	0.02 $\pm$ 0.08	0.06 $\pm$ 0.08	-0.01 $\pm$ 0.08	-0.01 $\pm$ 0.09	-0.21 $\pm$ 0.07	-0.22 $\pm$ 0.08
qP <sub>1900</sub>	0.26 $\pm$ 0.01	<b>0.28</b> $\pm$ 0.01	0.01 $\pm$ 0.05	-0.03 $\pm$ 0.07	0.08 $\pm$ 0.06	-0.03 $\pm$ 0.06	-0.35 $\pm$ 0.05	-0.42 $\pm$ 0.06
qN <sub>1900</sub>	0.88 $\pm$ 0.01	0.86 $\pm$ 0.001	0.02 $\pm$ 0.01	-0.02 $\pm$ 0.02	-0.01 $\pm$ 0.01	-0.02 $\pm$ 0.01	-0.06 $\pm$ 0.01	-0.03 $\pm$ 0.02
NPQ <sub>1900</sub>	<b>2.76</b> $\pm$ 0.07	2.42 $\pm$ 0.07	-0.06 $\pm$ 0.03	0.01 $\pm$ 0.04	-0.02 $\pm$ 0.03	0.01 $\pm$ 0.04	<b>-0.32</b> $\pm$ 0.05	-0.11 $\pm$ 0.05
$F_v/F_m$	0.76 $\pm$ 0.01	0.75 $\pm$ 0.01	-0.04 $\pm$ 0.02	-0.02 $\pm$ 0.02	-0.01 $\pm$ 0.01	-0.04 $\pm$ 0.01	-0.01 $\pm$ 0.01	0.03 $\pm$ 0.01
$\Phi_{\text{PSII}1900}$ ( $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$ )	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	-0.08 $\pm$ 0.08	0.03 $\pm$ 0.06	<b>-0.06</b> $\pm$ 0.05	0.15 $\pm$ 0.06	-0.22 $\pm$ 0.07	-0.32 $\pm$ 0.05
ETR <sub>1900</sub> ( $\mu\text{mol e}^- \text{ m}^{-2} \text{ leaf s}^{-1}$ )	71.03 $\pm$ 2.53	76.09 $\pm$ 2.99	-0.11 $\pm$ 0.08	-0.03 $\pm$ 0.06	<b>-0.09</b> $\pm$ 0.04	0.12 $\pm$ 0.06	-0.28 $\pm$ 0.05	-0.32 $\pm$ 0.05

LAR, leaf area ratio; LWR, weight ratio of leaf; RWR, weight ratio of root; SLA, specific leaf area; SWR, weight ratio of stem.



**Fig. 1.** Representation of biomass and survival profiles, (a) and (b), respectively. Significant differences found between invasive and native species within each treatment and within above- or below-ground biomass are denoted with an asterisk. Differences across treatment and invasive or native species were assessed by Tukey's post hoc and denoted by letters (above-ground biomass from a to e and below-ground biomass from g to j).

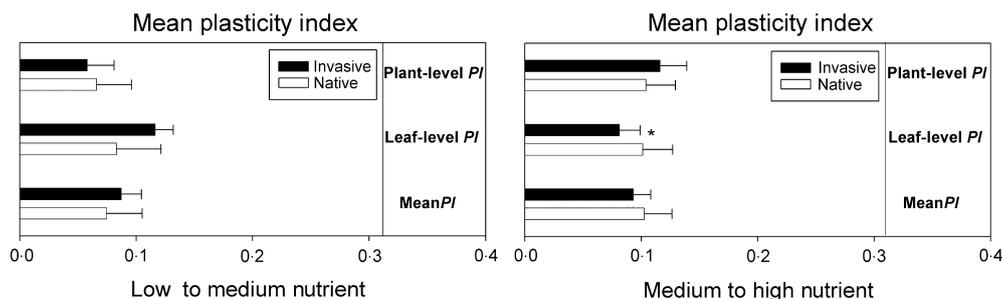
0.91,  $P = 0.83$ ], whereas invasive species had higher survival than natives under low light conditions [ $F(\text{light})_{1,119} = 14.22$ ,  $P < 0.01$ ;  $F(L/N)_{1,119} = 2.15$ ,  $P = 0.73$ ;  $F(\text{light} * L/N)_{1,119} = 17.32$ ,  $P < 0.01$  (Fig. 1)]. Finally, analysis including all variables showed that the effect of block and phylogenetic distance within pairs on survival were not significant either for the nutrient greenhouse [ $F(L/N)_{1,119} = 19.8$ ,  $P < 0.001$ ;  $F(\text{block})_{2,119} = 0.09$ ,  $P = 0.99$ ],  $F(\text{phylogeny})_{1,119} = 0.34$ ,  $P = 0.92$ , or for the light greenhouse [ $F(L/N)_{1,119} = 15.7$ ,

$P < 0.001$ ;  $F(\text{block})_{2,119} = 0.43$ ,  $P = 0.95$ ;  $F(\text{phylogeny})_{1,119} = 0.55$ ,  $P = 0.89$ ].

#### PHENOTYPIC PLASTICITY VALUES: INVASIVE VERSUS NATIVE

Trait plasticity in response to nutrient and light variation was highly variable within traits (e.g.  $N_{\text{area}} = 0.07\text{--}0.66$  or  $qP_{1900} = 0.01\text{--}0.42$ ) and between traits from low trait plasticity (e.g.  $F_v/F_m = 0.01\text{--}0.04$  or  $qN_{1900} = 0.01\text{--}0.06$ ) to high trait plasticity (e.g.  $LAR = 0.11\text{--}0.70$ ) (Table 2). However, relatively few traits showed significant differences in plasticity between invasive and native species. In most cases, variability in plant-level trait plasticity was captured by plant size (biomass as a covariable) in PERMANOVA analyses. Only after accounting for allometric effects, invaders did show significantly higher LAR plasticity from medium-to-high nutrient. For leaf-level traits, PNUE plasticity was consistently higher in invasive species from low-to-medium nutrient and from shade to sunlight.  $A_{\text{max}}$  followed the same pattern from low-to-medium nutrient availability. On the other hand, nitrogen content per mass ( $N_{\text{mass}}$ ) and per area ( $N_{\text{area}}$ ) varied less in invasive species from low-to-medium nutrient availability and from shade to sunlight conditions, respectively (Table 2). The effective quantum yield of photosystem II and electronic transport rate under at high irradiances ( $\Phi_{\text{PSII}1900}$ ,  $\text{ETR}_{1900}$ ) varied in opposite directions (i.e. the plasticity sign was different between groups) from medium-to-high nutrient (Table 2). While invasive species showed a positive increase in response to nutrient addition (i.e. negative  $PI_v$  values), native species did the opposite. Finally, PERMANOVA results for all trait-plasticity measurements, including into the analyses  $PI$  values from low-to-high nutrient, indicated that the effect of block and phylogenetic distances within pairs were not significant, either for nutrient gradient [ $F(L/N)_{1,119} = 22.8$ ,  $P < 0.001$ ;  $F(\text{nutrient})_{1,119} = 19.44$ ,  $P < 0.001$ ;  $F(\text{block})_{2,119} = 0.12$ ,  $P = 0.97$ ;  $F(\text{phylogeny})_{1,119} = 0.30$ ,  $P = 0.93$ ]; or for light gradient [ $F(L/N)_{1,119} = 20.72$ ,  $P < 0.001$ ;  $F(\text{block})_{2,119} = 0.66$ ,  $P = 0.90$ ;  $F(\text{phylogeny})_{1,119} = 0.55$ ,  $P = 0.89$ ].

The main pattern of nonsignificantly higher plastic responses of invaders was repeated for the mean of plant-level, leaf-level and overall plasticity in both nutrient and light gradients (Figs 2 and 3). In some cases, the invasive species even had lower phenotypic plasticity, as in the case of mean



**Fig. 2.** Nutrient gradient. Mean phenotypic plasticity of invasive and native species for plant-level traits, leaf-level traits and mean plasticity of the whole phenotype from low-to-medium nutrient level (left) and from medium-to-high nutrient level (right). \* $P < 0.05$ .

leaf physiology plasticity when nutrient availability increased from medium to high. In summary, contrary to theory, our results showed that invasive species did not display a higher phenotypic plasticity.

#### PHYLOGENETIC CONSERVATISM OF PLASTICITY

Cross-species phenotypic plasticity differences were not explained by the phylogenetic structure of the species selected when including together invasive and native species into the node-dated tree. All the taxonomic families showed the same level of phenotypic plasticity, across the phylogenetic tree; thus, no divergence or convergence between nodes was observed (plant-level plasticity  $r^2 = 0.09$ ,  $P = 0.80$ , leaf-level plasticity  $r^2 = 0.11$ ,  $P = 0.77$ , mean phenotypic plasticity  $r^2 = 0.04$ ,  $P = 0.91$ ) (Fig. 4).

## Discussion

#### MEAN PHENOTYPIC VALUES: INVASIVE VERSUS NATIVE SPECIES

Mean phenotypic values of traits related to growth and allocation are considered important for explaining invasiveness (Pyšek & Richardson 2007; van Kleunen, Weber & Fischer 2010). Several studies have shown that invasive species have lower root/shoot ratio and higher SLA and exhibit more efficient photosynthetic machinery compared with native or non-invasive species (Daehler 2003; Schlaepfer *et al.* 2010; Pyšek & Richardson 2007 and references therein, van Kleunen, Weber & Fischer 2010). At the same time, high resource-use efficiency (i.e. high carbon assimilation per unit of resource) has been hypothesized as a decisive feature allowing exotic species to become invasive in newly colonized ecosystems (Dukes & Mooney 1999; Niinemets, Valladares & Ceulemans 2003; Funk & Vitousek 2007). In support of both hypotheses, we have found significantly lower RWR and higher PNUE in invasive than in native species (Table 2). On the other hand, higher SLA and LAR as strong correlates of invasiveness (Daehler 2003; Hamilton *et al.* 2005) were not found in our

data. Although both groups had a similar rate of net photosynthesis per unit area ( $A_{max}$ ), invasive species achieved it with lower N investment in leaves ( $N_{area}$ ) (Table 2). Investing less nitrogen in leaves may be inherent to the nitrogen resource-use strategy of invasive species (Godoy *et al.* 2010). For instance, there were no significant differences in  $N_{mass}$  and  $N_{area}$  across the three nutrient levels (data not shown). Niinemets, Valladares & Ceulemans (2003) suggested that the origin of higher PNUE of invaders was related to reduction in soil nitrogen availability upon invasion because invasive species slow down nutrient cycling because of the production of litter with a high concentration of recalcitrant compounds (e.g. allelopathic and carbon-based compounds (Godoy *et al.* 2010). By contrast, Liao *et al.* (2008) and Ehrenfeld (2003) have found that most invasive species tend to speed up nutrient cycling, specially nitrogen-fixing invaders. Irrespective of what theory matches better with different empirical studies, our results support the idea that high PNUE is a key trait associated with invasiveness in both limiting and nonlimiting N environments.

Our results have documented for the first time the existence of a suite of traits that allow invasive species to achieve a higher light-harvesting efficiency than that of the natives and a better photosynthetic performance under a range of light environments. Significantly, higher light curve convexity ( $\Theta$ ) and lower light saturation point ( $I_c$ ) clearly enhanced carbon uptake of invasive species under limited irradiance (Table 2 and Fig. 1). Additionally, invaders avoided an excess of irradiance by producing higher amount of carbon-rich compounds, such as xanthophylls (significant higher  $NPQ_{1900}$ ), whereas native species tend to use photosynthesis pathway and thus chlorophylls (significant higher  $qP_{1900}$ ) (Table 2). These higher trait values associated with carbon acquisition by invaders and quenching differences between both groups suggest that invaders disposed enough carbon to diminish the carbon trade-off between growth and tissue protection (Villar *et al.* 2006). In contrast, native species might have to trade off growth for leaf photo-protection by producing N-rich pigments, such as chlorophylls, as suggested by their higher nitrogen leaf content ( $N_{area}$ ,  $N_{mass}$ ) (Table 2). Although we did not find significant differences in the physiological status of both groups (i.e.  $F_v/F_m$  was similar), this subtle difference between leaf photo-protection strategies involves for native species an increased risk of damage of their chlorophylls by photo-inhibition, which in turn can decrease carbon gain and growth.

Collectively, our results reveal that the invasive species studied displayed high tolerance to and high performance over a wide range of nutrient and light conditions. This is in agreement with results for other ecosystems (Mooney & Hobbs 2000; van Kleunen, Weber & Fischer 2010). The higher trait mean values for key aspects of physiological performance of invasive species must positively influence their capacity to outcompete natives. These higher trait means might be more important than phenotypic plasticity to succeed under changing conditions because in Mediterranean-type ecosystems (MTE), plasticity is not always

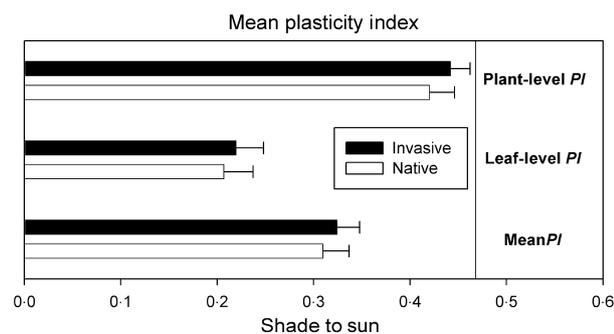


Fig. 3. Light gradient. Mean phenotypic plasticity of invasive and native species for plant-level traits, leaf-level traits and mean plasticity of the whole phenotype from shade to sun. \* $P < 0.05$ .

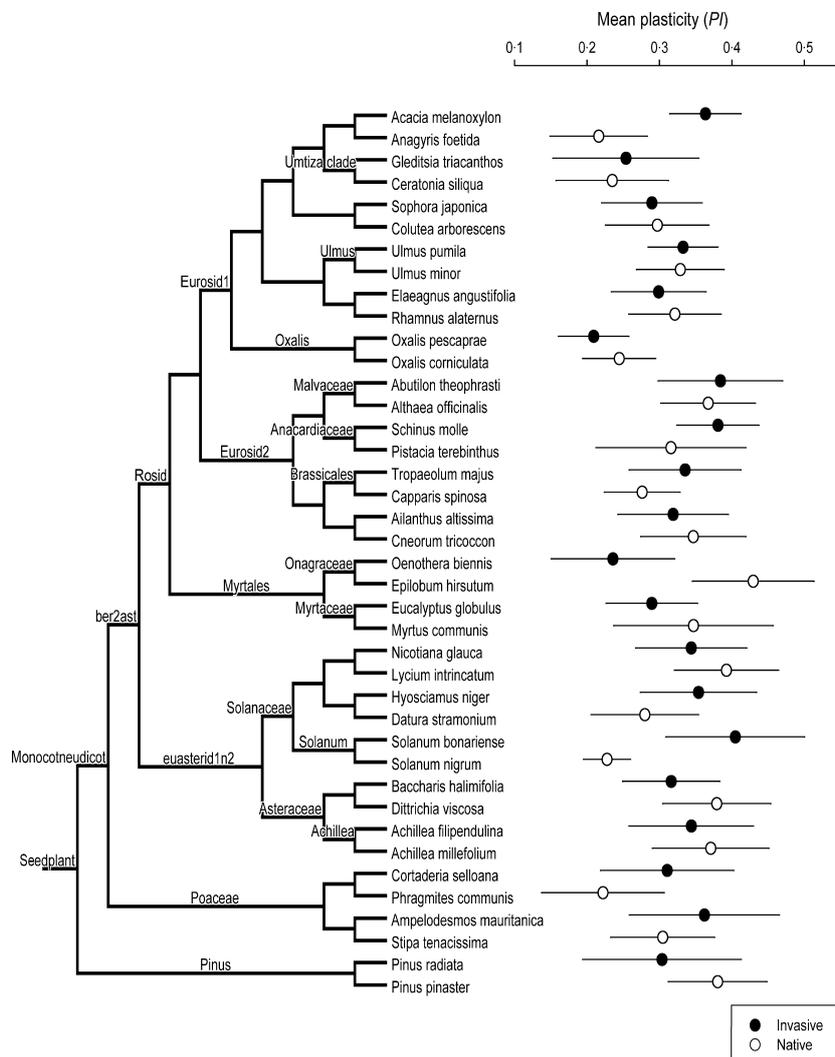


Fig. 4. Phylogenetic tree of the mean plasticity across invasive and native species. Mean  $\pm$  SE for each species is also shown.

advantageous. Under stochastic and unpredictable resource fluctuations, plastic adaptive responses to a given abiotic factor can turn out to be maladaptive when another abiotic factor is also fluctuating and stressful (see discussion in Valladares, Gianoli & Gomez 2007).

#### PHENOTYPIC PLASTICITY: INVASIVE VERSUS NATIVE SPECIES

Our results do not match with previous studies that suggest that invasiveness of exotic species is related to an increased phenotypic plasticity (see for instance Gerlach & Rice 2003; Niinemets, Valladares & Ceulemans 2003; Davidson, Jennions & Nicotra 2011). In general, the level of plasticity of invasive species measured at multiple scales (trait, mean trait-level and overall mean plasticity) was similar, and in some cases even lower, to that of natives (Figs 2 and 3, Table 2). In addition, both invasive and native species had highly plastic responses across the entire resource gradient, supporting the

idea that trait plasticity is not constrained in low resource environments (Funk 2008). Still, our results should be corroborated at very low resource conditions where species usually show nonlinear plastic responses (e.g. light availability below 20% full radiation) (Poorter 1999). The lack of evidences supporting high plasticity as a determinant of invasiveness might indicate that their importance *per se* is low compared with other mechanisms. For instance, invasiveness may be also attributed to the benefits of escaping from natural enemies, such as pathogens or predators, as predicted by the enemy release hypothesis (ERH) and the evolution of increased competitive ability (EICA). As there are almost 20 different hypotheses in the literature trying to explain invasiveness, future promise research may be channelled to distinguishing the relative importance of different hypotheses.

Even so, invasive species did display higher plasticity for a few traits under particular resource availabilities, providing some insights into plastic strategies that may convey invasiveness. When resource availability changed from low to

medium nutrient and from shade to sun, invasive species displayed higher plasticity in only two leaf physiological traits, PNUE and N leaf content (both  $N_{\text{mass}}$  and  $N_{\text{area}}$ ) (Table 2). Funk (2008) investigated the plastic responses of five invasive–native pairs in low resource environments of the Hawaiian Islands and found that  $A_{\text{max}}$  and  $N_{\text{area}}$  were positively related to the invasive species fitness, although this pattern was observed in response to nitrogen but not to light availability. Thus, it seems that high plasticity in leaf-level nitrogen and light-use traits is important for successful plant invasions, particularly in low resource environments.

Surprisingly, when resource conditions change from medium to high nutrient level, invasive species tracked the nutrient increase with a higher production of leaf area per unit biomass (plasticity for LAR) than natives, rather than increasing  $N_{\text{mass}}$  in leaves (Table 2). Perhaps, higher LAR plasticity entailed higher plasticity in  $\Phi_{\text{PSII}}$  and ETR at high light intensities,  $1900 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 2), because new leaves have their photosynthesis machinery intact. If this is the case, the production of new leaves to maximize carbon acquisition may be a profitable strategy to grow faster when nitrogen is abundant. Overall, these plastic responses support the idea of invader's fast-growing dynamics as suggested by Niinemets, Valladares & Ceulemans (2003), for example.

#### RELATIONSHIP BETWEEN TRAITS, PLASTICITY AND FITNESS

Strong trait variation responses do not necessarily confer success to exotics; instead the interaction of plasticity with certain trait values (typically high values) results in a 'general purpose phenotype' (i.e. high mean values of traits associated with a strong ability to compete along broad ranges of environmental conditions). Following the predictions of Richards *et al.* (2006), our results showed that invasive species can be classified in different categories depending on how resource gradients affect fitness traits. While in the nutrient gradient, invasive species followed the 'Master-of-some' strategy because they showed similar levels of survival to natives across nutrient treatments but with higher biomass as nitrogen availability was increased, and in the light gradient, invaders followed a 'Jack-and-Master' strategy because they always had higher levels of above-ground biomass and higher rate of survival than natives in shade (Fig. 1).

Further attempts are needed to distinguish the mechanism by which invasive species benefit more from plasticity than natives. Theoretically, two potential mechanistic explanations (not mutually exclusive) can explain this result: (i) invasive species display high trait plasticity resulting in significantly higher fitness than the natives, and (ii) invasive species have the same plasticity level but trait values associated with fitness were always higher in invasive species. Our results of similar plasticity between both groups but higher capacity for carbon acquisition by invasive species suggest that the latter mechanism explains the higher fitness of invasive species. However, this might not be always the case. Further studies applying multivariate techniques such as

structural equation modelling (SEM, see Shipley 2004 for details) should explicitly disentangle the relative importance for plant fitness of trait mean value, phenotypic plasticity and other important features not measured in this study such as phenotypic integration (Murren, Pendleton & Pigliucci 2002).

#### PHYLOGENETIC SIGNAL

The lack of phylogenetic signal in trait plasticity across different plant scales (leaf-level, whole plant-level and mean phenotypic plasticity) suggests that there are no significant phylogenetic constraints for wide trait variation (Fig. 4). In addition, phylogenetic analyses suggest that higher levels of phenotypic plasticity are not related to a specific growth form of invasive species, because no differences were found at the family-level nodes where woody and herbaceous are nested to each taxonomic family. Therefore, these results reflect the fact that plasticity is a convergent evolutionary strategy, at least for the set of species studied here.

#### STRENGTHS AND LIMITATIONS OF THE STUDY

Multispecies comparisons enable more robust conclusions than single-species studies, but they have been restricted to the evaluation of a reduced number of traits (Goodwin, McAllister & Fahrig 1999; Prizing *et al.* 2002; van Kleunen, Johnson & Fischer 2007). Very few studies have tackled the invasiveness of exotic species experimentally with a large number of species and traits (van Kleunen & Johnson 2007; Schlaepfer *et al.* 2010). However, species selection is an important step in multispecies comparison studies (van Kleunen *et al.* 2010), and our study has the limitation that three native species, *Pinus pinaster*, *Achillea millefolium* and *Diitrichia viscosa*, were also recorded as invasive species elsewhere (Wacquand 1990; Rejmánek & Richardson 1996; Beckmann, Erfmeier & Bruelheide 2009). Besides, methods to control for ontogenetic drift were only partial because plant biomass did not overlap between treatments. We acknowledge that both facts, invasiveness elsewhere and ontogenetic drift, might have affected our results. Despite these limitations, our study provides novel insights on invasiveness and an important methodological conclusion: phenotypic plasticity should not be considered alone regarding invasiveness, but rather in combination with trait mean values.

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## Supporting Information

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

**Appendix S1.** Method for controlling partially plasticity with ontogeny.

**Appendix S2.** Measurements of leaf fluorescence.

**Table S1.** Invasive-Native pairs selected for the experiment.

**Table S2.** Trait mean values detailed for each species.

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